Annales
de la
Société
Entomologique
de
France

The cereal stem borers of Sub-Saharan Africa and their antagonists
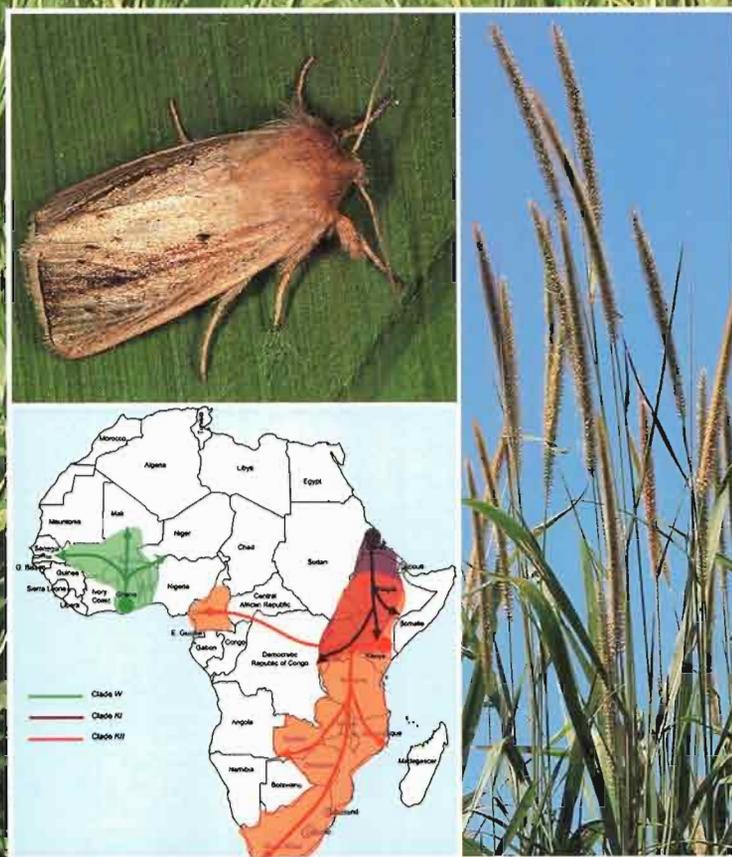
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2006, 42 (3-4)

ANNALES
DE LA SOCIÉTÉ ENTOMOLOGIQUE DE FRANCE
(Nouvelle série)

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Volume 43 2007

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6 janvier 1947 - 2 juillet 2006

Directeur de la Publication des Annales de la Société Entomologique de France

Hommage de la Rédaction

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Les recherches sur les lépidoptères foreurs des graminées et leurs antagonistes : bilan et perspectives

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Abstract. Research on the lepidopteran graminaceous stem borers and their antagonists: achievement and perspectives. In sub-Saharan Africa, lepidopteran stem borers cause severe damage and yield loss to graminaceous crops such as maize, sorghum and sugarcane. This paper reports major findings on systematics, populations genetics and stem borer ecology by scientists from France, Australia and several African countries, over the last five years. Some of these findings do not agree with past results, which will affect our approach to control stem borers. These articles will be an important source of information for decision makers, scientists and extension agents working on stem borers.

Résumé. Les Lépidoptères foreurs des tiges constituent un des principaux facteurs de réduction de la production de graminées cultivées (maïs, sorgho et canne à sucre) en Afrique sub-saharienne. Une synthèse des recherches menées au cours des cinq dernières années sur ces insectes ravageurs et leurs antagonistes est présentée. Les travaux réalisés dans ce domaine par des équipes françaises, australiennes et de plusieurs pays d'Afrique ont conduit à un ensemble de résultats originaux dans les domaines de la systématique, de la génétique des populations et de l'écologie des foreurs. Ces résultats bouleversent significativement les connaissances acquises antérieurement, et par là même devraient conduire dans les prochaines années à un changement des méthodes de gestion de ces ravageurs. Les travaux faisant l'objet de la présente synthèse apportent aux chercheurs, décideurs et acteurs de terrain une information actualisée sur les foreurs des graminées, information difficilement accessible par ailleurs.

Keywords: Africa, maize, sorghum, stem borers, parasitoids, predators.

L'homme agit de façon croissante sur l'environnement et les systèmes écologiques. En retour, ces systèmes vont répondre via des mécanismes propres et interférer avec les activités humaines. Il est donc essentiel de comprendre ces mécanismes.

Plus de la moitié des êtres vivants dans le monde sont des insectes et environ la moitié d'entre eux a un régime phytophage, c'est-à-dire qu'ils consomment différentes parties des plantes, feuilles, tiges, racines, fleurs, fruits ou graines. La phytophagie est répandue dans les principaux ordres d'insectes, mais, parmi les ordres les plus riches en espèces, les lépidoptères comprennent une très forte proportion d'espèces phytophages. Cette phytophagie s'exerce principalement aux dépens des plantes à fleurs, les Angiospermes. Les lépidoptères constituent donc un élément clef des biocénoses et il n'est pas étonnant qu'ils interfèrent fréquemment avec l'homme en tant que ravageurs, en particulier dans les régions tropicales où se concentre une forte proportion de la diversité entomologique. En Afrique, par exemple, l'homme a introduit des plantes cultivées qui n'en étaient pas originaires (arachide, manioc, maïs, etc.), ou a domestiqué des espèces végétales locales pour créer une variété ou une espèce cultivée dont il a intensifié la culture (e.g. *Sorghum bicolor*). Dans certains cas des insectes ont été transportés avec ces plantes (cas de *Chilo partellus* (Swinhoe 1885) (Lepidoptera : Crambidae) important ravageur du maïs originaire d'Asie et introduit accidentellement en Afrique vers 1930) se sont adaptés à de nouveaux biotopes et, en l'absence de compétiteurs locaux, ont pu devenir des espèces envahissantes. Dans d'autres cas, ce sont des insectes locaux qui se sont adaptés à une ressource végétale domestiquée ou nouvellement introduite, comme dans le cas du foreur *Busseola fusca* (Fuller 1901) (Lepidoptera : Noctuidae) vis-à-vis du sorgho et du maïs, par exemple.

En Afrique, les lépidoptères foreurs, dont les premiers stades larvaires se nourrissent directement de feuilles pour ensuite forer les tiges, constituent l'une de principales contraintes de production des céréales cultivées. Leur diversité apparaît toutefois beaucoup plus importante qu'on ne le considérait jusqu'à présent (Le Rü *et al.* 2006). Ce nouvel essor de l'étude de la diversité spécifique des foreurs est probablement lié à l'utilisation accrue de l'outil moléculaire qui, combiné à l'étude morphologique classique des espèces permet de mieux définir le statut taxonomique du matériel récolté (Moyal 2006). Beaucoup de ces foreurs se sont spécialisés sur une espèce végétale (monophagie) ou un petit nombre d'espèces appartenant à la même famille botanique (oligophagie) (Le Rü *et al.* 2006 ; Ong'amo *et al.* 2006 ; Otieno *et al.* 2006). Une meilleure compréhension des mécanismes de sélection et d'adoption d'une plante hôte est illustrée par l'exemple de l'étude de l'équipement sensoriel de *B. fusca* (Calatayud *et al.* 2006). La phytophagie et la spécialisation sur certaines espèces d'angiospermes sont vraisemblablement à l'origine du succès évolutif de ces lépidoptères foreurs de graminées en Afrique (Moyal & Le Rü 2006).

Les interactions entre les foreurs actuels et leurs plantes hôtes peuvent être très anciennes et ont été conservées au cours du temps (Moyal & Le Rü 2006). Ce conservatisme des interactions plantes-foreurs implique que des contraintes fortes jouent sur les phénomènes de changements d'hôte végétal, facteurs majeurs de la spéciation et donc de la diversification de ces insectes phytophages.

Des études phylogéographiques ont mis en évidence une variabilité génétique des populations de certaines espèces de foreur comme *Eldana saccharina* Walker 1865 (Lepidoptera : Pyralidae), un important ravageur de la canne à sucre en Afrique du Sud, et *B. fusca* en fonction de leur origine géographique (Assefa *et al.* 2006 ; Sezonlin *et al.* 2006). Ces travaux permettent de retracer l'histoire de la distribution géographique et écologique des populations de ces espèces au cours du temps sur le continent Africain.

L'étude des phénomènes de spéciation chez les foreurs requiert également une bonne connaissance des mécanismes de reconnaissance du partenaire sexuel. Chez *B. fusca,* espèce chez laquelle la femelle émet une phéromone sexuelle qui assure l'attraction spécifique des mâles, les études comportementales ont montré que ces derniers présentaient un comportement de cour extrêmement simplifié (Frérot *et al.* 2006).

Si les lépidoptères foreurs de graminées semblent s'être diversifiés en exploitant la diversité des plantes (Le Rü *et al.* 2006), la compréhension de leur histoire évolutive implique aussi la prise en compte des organismes qui vont tenter de réguler leurs populations, comme les insectes parasitoïdes. Les lépidoptères foreurs sont engagés dans des processus coévolutifs avec leurs antagonistes (Branca & Dupas 2006 ; Bruce *et al.* 2006 ; Conlong & Goebel 2006 ; Dittrich *et al.* 2006 ; Dupas *et al.* 2006 ; Gitau *et al.* 2006 ; Muirhead *et al.* 2006 ; Wale *et al.* 2006), processus où les facteurs de l'environnement, la plante hôte, les autres espèces hôtes potentielles des antagonistes et la contamination par *Wolbachia pipientis* Hertig, 1936 (Riketsiales : Rickettsiaceae), jouent un rôle prépondérant. Une meilleure connaissance de ces processus évolutifs et de leur action sur la démographie des hôtes doit permettre une meilleure gestion et utilisation de ces antagonistes pour le contrôle des populations de lépidoptères foreurs ravageurs de culture.

Le parfait synchronisme spatio-temporel qui existe entre certaines espèces de foreurs et de parasitoïdes (Jiang *et al.* 2006) a permis une utilisation intensive de *Cotesia flavipes* Cameron 1891 (Hymenoptera : Braconidae) pour contrôler efficacement les populations de *C. partellus* sur maïs en Afrique (Cugala *et al.* 2006 ; Kipkoech *et al.* 2006 ; Omwega *et al.* 2006). Cette espèce est également considérée comme le meilleur candidat pour un contrôle biologique des foreurs en cas d'invasion des plantations de canne à sucre en Australie (Sallam 2006).

Différentes pratiques culturales comme l'utilisation de plantes en bordure des champs cultivés (haies protectrices), la fertilisation du sol et les cultures associées se sont avérées prometteuses pour réduire les dégâts et les pertes de rendement causés par les lépidoptères foreurs (Agboka *et al.* 2006 ; Ali *et al.* 2006 ; Chabi-Olaye *et al.* 2006 ; Matama-Kauma *et al.* 2006 ; Mgoo *et al.* 2006 ; Ndemah *et al.* 2006 ; Van den Berg 2006).

Par rapport à 1996, date de la dernière conférence internationale sur les lépidoptères foreurs de graminées en Afrique, qui s'était tenue à Nairobi, à l'ICIPE (Overholt 1997), les résultats présentés dans ce volume ont permis de renouveler très profondément les connaissances dans les domaines de la diversité faunistique, de la génétique des populations, de la distribution spatio-temporelle et de la biologie des foreurs de tiges et de leurs antagonistes. Les résultats obtenus au cours des dix dernières années doivent aussi permettre une amélioration de l'efficacité des méthodes de contrôle biologiques et culturales. L'amélioration des connaissances dans ces domaines permettra à long terme le développement d'un ou plusieurs modèles de prédiction des fluctuations des populations de foreurs, prenant en compte la diversité

des conditions environnementales locales. De tels modèles pourront, par exemple, aider à une meilleure gestion de l'introduction et de l'utilisation du maïs transgénique en Afrique.

Références

Agboka K., Gounou S., Tamò M. 2006. The role of maize-legumes-cassava intercropping in the management of maize ear borers with special reference to *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae). *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 495-502.

Ali A.I., Jiang N., Schulthess F., Ogol C.K.P.O., Omwega C.O. 2006. Effect of nitrogen fertilizer level on infestations of lepidopterous stemborers and yields of maize in Zanzibar. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 481-486.

Assefa Y., Conlong D.E., Mitchell A. 2005. Phylogeography of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Annales de la Société Entomologique de France* **42(3-4):** 331-337.

Branca A., Dupas S. 2006. A model for the study of *Wolbachia pipientis* Hertig (Ricketsiales: Rickettsiaceae) induced cytoplasmic incompatibility in arrhenotokous haplodiploid populations: consequences for biological control. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 443-448.

Bruce A.Y., Schulthess F., Mueke J. 2006. Factors affecting the bionomics of the eastern African egg parasitoid *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae). *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 423-431.

Calatayud P.-A., Chimtawi M., Tauban D., Marion-Poll F., Le Rü B.P., Silvain J.-F., Frérot B. 2006. Sexual dimorphism of antennal, tarsal and ovipositor chemosensilla in the African stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 403-412.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2006. Relationships of soil fertility and stem borers damage ro yield in maize-based cropping system in Cameroon. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 471-479.

Conlong D.E., Goebel F.R. 2006. *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae) and *Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae) in sugarcane in Mozambique- A new association. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 417-422.

Cugala D., Schulthess F., Ogol C.P.O., Omwega C.O. 2006. Assessment of the impact of natural enemies on stem borer infestations and yield loss in maize using selected insecticides in Mozambique. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 503-510.

Dittrich G., Conlong D.E., Mitchell A. 2006. Genetic Diversity of *Sturmiopsis parasitica* Curran (Diptera: Tachinidae). *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 325-329.

Dupas S., Gitau C., Le Rü B.P., Silvain J.-F. 2006. Single-step PCR differentiation of *Cotesia sesamiae* (Cameron) and *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) using polydnavirus markers. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 319-323.

Frérot B., Félix A.-E., Ene S., Calatayud P.-A., Le Rü B.P., Guenego H. 2006. Courtship behaviour of the African maize Stem Borer: *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) under laboratory conditions. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 413-416.

Gitau C.W., Dupas S., Ngi-Song A.J., Mbugi J.P., Schulthess F. 2006. Calyx fluid proteins of two *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) biotypes in Kenya: implications to biological control of the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 433-441.

Jiang N., Zhou G., Overholt W.A., Muchugu E., Schulthess F. 2006. The temporal correlation and spatial synchrony in the stemborers and parasitoid system of Coast Kenya with climate effects. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 381-387.

Kipkoech A.K., Schulthess F., Yabann W.K., Maritim H.K., Mithöfer D. 2006. Biological control of cereal stem borers in Kenya: A cost benefit approach. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 519-528.

Le Rü B.P., Ong'amo G.O., Moyal P., Muchugu E., Ngala L., Musyoka B., Abdullah Z., Matama-Kauma T., Lada V.Y., Pallangyo B., Omwega C.O., Schulthess F., Calatayud P.-A., Silvain J.-F. 2006. Geographic disrribution and host plant ranges of East African noctuid stem borers. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 353-361.

Matama-Kauma T., Schulthess F., Mueke J.M., Omwega C.O., Ogwang J.A. 2006. Effect of wild grasses planted as border rows on stem borer infestations in maize in Uganda. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 455-460.

Mgoo V.H., Makundi R.H., Pallangyo B., Schulthess F., Jiang N., Omwega C.O. 2006. Yield loss due to the stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) at different nitrogen application rates to maize. *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 487-494.

Moyal P. 2006. History of the systematics of the *Sesamia sensu lato* group of African noctuid stem borers of monocotyledonous plants (Lepidoptera). *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 285-291.

Moyal P., Le Rü B.P. 2006. From population to species: morphological and molecular diversity in east African stem borer species of the genus *Manga* Bowden 1956 (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 293-307.

Muirhead K.A., Murphy N.P., Sallam M.N., Donnellan S.C., Austin A.D. 2006. Mitochondrial DNA phylogeography of the *Cotesia flavipes* complex of parasitic wasps (Hymenoptera: Braconidae). *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 309-318.

Ndemah R., Schulthess F., Nolte C. 2006. The effect of grassy field margins and fertilizer on soil water, plant nutrient levels, stem borer attacks and yield of maize in the humid forest zone of Cameroon. *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 461-470.

Omwega C.O., Muchugu E., Overholt W.A., Schulthess F. 2006. Release and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) an exotic parasitoid of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in East and Southern Africa. *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 511-517.

Ong'amo G.O., Le Rü B.P., Dupas S., Moyal P., Muchugu E., Calatayud P.-A., Silvain J.-F. 2006. The role of wild host plants in the abundance of lepidopteran stem borers along altitudinal gradient in Kenya. *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 363-370.

Otieno N.A., Le Rü B.P., Ong'amo G.O., Dupas S., Calatayud P.-A., Makobe M., Ochora J., Silvain J.-F. 2006. Diversity and abundance of wild host plants of lepidopteran stem borers in two different agroecological zones of Kenya, Kakamega and Muhaka. *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 371-380.

Overholt W.A. 1997. Ecology and management of tropical gramineous stemborers. Proceedings of the International Workshop on the Management of Tropical Gramineous Stemborers held in Nairobi, ICIPE, 14-16 May 1996. *Insect Science and Its Application* 17(1): 1-173.

Sallam M.N.S. 2006. A review of sugarcane stem borers and their natural enemies in Asia and Indian Ocean Islands: an Australian perspective. *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 263-283.

Sezonlin M., Dupas S., Le Rü B.P., Faure N., Le Gall P., Silvain J.-F. 2006. Phylogeographic pattern and regional evolutionary history of the maize stalk borer *Busseola fusca* (Fuller) (Lepidoptera, Noctuidae) in sub-Saharan Africa. *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 339-351.

Van den Berg J. 2006. Vetiver grass (*Vetiveria zizanioides* (L.) Nash) as trap plant for *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 449-454.

Wale M., Schulthess F., Kairu E.W., Omwega C.O. 2006. Distribution and relative importance of cereal stem borers and their natural enemies in the semi-arid and cool-wet ecozones of the Amhara State of Ethiopia. *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 389-402.

A review of sugarcane stem borers and their natural enemies in Asia and Indian Ocean Islands: an Australian perspective

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Abstract. This paper provides a review on lepidopteran stem borer pests of graminaceous crops in Asia and Indian Ocean Islands which have the potential to invade Australia. Information on the geographical distribution, host plants and potential of invading Australia is provided for 36 stem borer species. A literature review of all natural enemies of 18 key pest species is provided. A knowledge of possible biological control options is essential to determine which natural enemies are to be considered for introduction following an incursion. The Braconid, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), stands out as a promising candidate for introduction into Australia following a borer incursion. Studies are currently being conducted on a native *Cotesia* species in Australia, which may be able to parasitize larvae of exotic borers, therefore minimizing the need for other parasitoids introductions.

Résumé. Revue des foreurs de la canne à sucre et de leurs enemis naturels en Asie et dans les îles de l'Océan indien : une perspective australienne. Cet article passe en revue les lépidoptères foreurs de tiges de graminées cultivées en Asie et dans les îles de l'Océan Indien, et susceptibles d'envahir l'Australie. Des données sur la distribution géographique, les plantes hôtes et les espèces potentiellement invasives de l'Australie sont présentées pour 36 de ces espèces. Une revue bibliographique de tous les ennemis naturels associés à 18 ravageurs majeurs est fournie. Une telle connaissance des ennemies naturels est nécessaire au préalable afin de proposer les agents biologiques nécessaires à introduire en cas d'invasion d'un ravageur exotique. Le Braconide *Cotesia flavipes* Cameron (Hymenoptera : Braconidae), apparaît être comme un des candidats les plus prometteurs en cas d'une telle invasion. Des études sont actuellement conduites en Australie sur une espèce indigène de *Cotesia* qui serait capable de parasiter des larves de foreurs exotiques, ce qui réduirait la nécessité d'introduction de parasitoïdes eux-mêmes exotiques.

Keywords: Stem borers, sugarcane, Australia, natural enemies, *Chilo, Sesamia, Scirpophaga, Maliarpha, Acigona, Argyroploce, Cotesia.*

Lepidopterous stem borers are major pests of graminaceous crops in most countries of the world. Fortunately, Australia does not harbour major borer species, however, several key stem borers are widely distributed in neighbouring countries. The incursion of any of these pests into Australia would result in severe consequences to the Australian sugar industry. In attempt to be prepared for possible borer incursion into Australia, there is a need to identify borer species and their natural enemies in neighbouring countries. This knowledge is required to recognize the most suitable natural enemy for importation into Australia in case of incursion (Allsopp *et al.* 2000).

Several successful attempts of classical biological control (CBC) of graminaceous stem borers are well documented, such as the notable success of the establishment of *Cotesia flavipes* Cameron

(Hymenoptera: Braconidae) in East Africa and Indian Ocean islands on a range of stem borer species (Rajabalee & Governdasamy 1988; Overholt *et al.* 1997). However, in South Africa, 13 species of parasitoids were introduced over 16 years to control a complex of borer species but none has established (Kfir 1997). Hence, studying the geographical distribution and host range of a natural enemy is required prior to its introduction. This paper provides a list of all stemborers recorded to feed on sugarcane in the world. A ranking system was followed to assess their threat to Australia. If a borer species is geographically close to Australia and on the same time regarded as a major pest where it is distributed then it is ranked "high threat"; if it only fulfils one of these two conditions then it is regarded a "medium threat"; while if it does not fulfil any of the two then it is ranked "low threat". This paper also reviews the distribution of borer species in Asia and Indian Ocean islands with the potential to invade Australia, and provides a catalogue of their old and new-association natural enemies recorded over the

past 100 years. Other species such as *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) and *Diatraea* spp. are major pests in Africa and central and South America respectively. Information on biological control of *E. saccharina* can be found in Carnegie *et al.* (1985) and Conlong (1997). Information on biological control of *Diatraea* spp. is available in Rodriguez-del-Bosque *et al.* (1990), Smith *et al.* (1993) and Smith (1994). In Australia, only two minor borer species attack sugarcane and are rarely seen in cane fields, and these are the noctuid *Bathytricha truncata* (Walker), and the gelechiid *Ephysteris promptella* (Staudinger) (Jarvis 1927; Jones 1966). Bell (1934) reports *Apanteles flavipes* Cameron (*nonagriae* Ol. & Vier) (Hymenoptera: Braconidae) as a larval parasitoid on the former species, while no natural enemies are recorded on the latter.

Table 1 lists all borer species recorded to attack sugarcane in the world, with information on their host range and geographical distribution. Table 2 lists all records made of natural enemies of graminaceous stem borers only in Asia and Indian Ocean islands. References were numbered from 1 – 154; the species name and country of record where followed by the reference number in cases of multiple entries or where plant hosts included crops other than sugarcane, and a note is made on the status and origin of the enemy where relevant. Natural enemies recorded to exploit a host only in the laboratory were not included. Pests such as *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) are widely distributed in main land Africa, while others such as *Sesamia cretica* Ledere (Lepidoptera: Noctuidae) extend to Southern Europe, but only natural enemies recorded in Asia and Indian Ocean islands are presented. Information on their natural enemies in main land Africa can be found in Polaszek (1998).

Out of 36 borer species recorded to attack sugarcane, 7 are regarded "high threat", 15 were "medium threat" and 14 were regarded "low threat" to Australia. Table 2 lists a number of 276 natural enemy species of 18 key pests of graminaceous plants. That list indicates that the majority of species recorded as biological control agents of stem borers in Asia are mainly native, and that successful CBC attempts were limited to only a few number of introductions. Two main parasitoids had the highest number of recorded introductions and establishments, and these are *Co. flavipes* and *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae). Based on this work and several previous studies, *Co. flavipes* stands out as an efficient natural enemy of most stem borers in neighbouring countries. According to table 2, *Co. flavipes* is capable of parasitizing 15 out of 18 stem borer pest species distributed in Asia and Indian Ocean islands. Though there are no records of *Co. flavipes* attacking *S. calamistis* in Mauritius, the parasitoid is recorded on that host in mainland Africa, in addition, other *Chilo* species

such as *Chilo orichalcociliellus* (Strand) (Lepidoptera: Crambidae) are also attacked by *Co. flavipes* in corn in main land Africa (Ngi-Song *et al.* 1995). *Co. flavipes* is also recorded to parasitize a wide range of borer species of the New World genus *Diatraea* (Rodriguez-del-Bosque *et al.* 1990). However, the record of *Apanteles* (*Cotesia*) *flavipes* Cam. (Hymenoptera: Braconidae) on *Scirpophaga excerptalis* Walker (Lepidoptera: Crambidae) is doubtful as the female is incapable of reaching host larvae inside the growing point, though may sting the host in the laboratory (Sallam, personal observation).

A range of natural enemies attacking different host stages may be needed to successfully control a target pest (Smith *et al.* 1993; Smith & Wiedenmann 1997). Primarily, knowledge of the endemic natural enemy complex attacking an introduced pest in the country it invaded is required to identify which host stage is to be targeted for natural enemy introduction. For example, introducing egg parasitoids into South Africa had no impact on *E. saccharina* populations, since a large proportion of eggs and neonate larvae is already eaten by predators (Conlong 1997). This agrees with van Hamburg & Hassell (1984), who showed that the impact of an additional mortality factor that targets a stage with already high natural mortality is negligible. Alternatively, the pupal parasitoid, *X. stemmator* was introduced to Mozambique, where *C. sacchariphagus* was first confirmed in 1999, while no indigenous parasitoids were recorded (Way & Turner 1999). Post release surveys showed a sharp reduction in the host population in all release fields (Conlong & Goebel 2002). Table 2 shows that *X. stemmator* is recorded on 8 key stem borers, therefore may act as an important candidate for introduction to Australia in case of incursion by any of its hosts. No direct competition between *Co. flavipes* and *X. stemmator* is expected as they attack different host stages and use different attack strategies.

In Australia, the name *Apanteles nonagriae* Olliff. nec Viereck (Hymenoptera: Braconidae) is cited as a synonym of *Apanteles* (*Cotesia*) *flavipes* Cam. (Hymenoptera: Braconidae) (Austin & Dangerfield 1992), however the two could be sibling species. The occurrence of *Co. flavipes* in Australia is an area that requires more studies. The wide host range of *Co. flavipes* qualifies it to be a strong candidate in case of incursion by key borers into Australia. Whether the Australian population is capable of exploiting the exotic borers or there is need to introduce another population is a point worth investigating.

Table 1. Level of threat to Australia by world moth borer species recorded to attack sugarcane.

Species	Family	Major host plant (s)	World distribution	Threat to Australia
Chilo infuscatellus Snellen	Crambidae	Sugarcane, wide range of gramineous crops	Northern, central and South East Asia	High
Chilo auricilius Dudgeon	Crambidae	Sugarcane, rice	Central and South East Asia	High
Scirpophaga excerptalis Walker	Crambidae	Saccharum spp., range of wild grasses	Central ro South East Asia, Indonesia and PNG	High
Chilo sacchariphagus (Bojer)	Crambidae	Sugarcane, sorghum	Central to South East Asia. Indian ocean islands	High
Sesamia inferens Walker	Noctuidae	Sugarcane, wide range of gramineous crops	Japan, cental and South East Asia, Indonesia and PNG	High
Sesamia grisescens Warren	Noctuidae	Saccharum spp., range of wild grasses	Restricted to PNG	High
Chilo terrenellus Pagenstecher	Crambidae	Saccharum spp.	PNG, Vulcan island, Torres Strait island of Saibei	High
Eldana saccharina Walker	Pyralidae	Sugarcane, Maize	Sub Saharan Africa	Medium
Diatraea saccharalis (Fabricius)	Crambidae	Sugarcane, corn, sorghum	The Caribbean, Central America. Southern USA	Medium
Eoreuma loftini (Dyar)	Crambidae	Sugarcane, corn, sorghum	Mexico, southern Texas	Medium
Chilo partellus (Swinhoe)	Crambidae	Corn, sorghum, rice, cane	Central and South East Asia, sub Saharan Africa	Medium
Chilo tumidicostalis (Hampson)	Crambidae	Sugarcane	India, South East Asia	Medium
Diatraea rosa Heinrich	Crambidae	Sugarcane	Venezuela	Medium
Chilo polychrysus (Meyrick)*	Crambidae	Rice, maize	Central and South East Asia, Northern Territory (?)	Medium
Scirpophaga magnella (de Joannis)	Crambidae	Saccharum spp.	Central and South East Asia	Medium
Diatraea considerata Heinrich	Crambidae	Sugarcane	Mexico and Venezuela	Medium
Emmalocera depressella (Swinhoe)	Pyralidae	Sugarcane	India, Pakistan, Bangladesh	Medium
Diatraea centrella (Moschulsky)	Crambidae	Sugarcane	West Indies, Guyana, Surinam, French Guiana, Venezuela	Medium
Sesamia calamistis Hampson	Noctuidae	Maize, wide range of gramineous crops	Sub Saharan Africa , Indian Ocean islands	Medium
Sesamia cretica Lederer	Noctuidae	Maize, cane, gramineous crops, tomatoes	Southern Europe, North Africa, Central Asia	Medium
Diatraea busckella Dyar & Heinrich	Crambidae	Sugarcane	Colombia, Venezuela	Medium
Tetramoera schistaceana (Snellen)	Tortricidae	Sugarcane	Japan, Central and South East Asia, Indian Ocean islands	Medium
Acigona steniellus Hamp	Pyralidae	Sugarcane	India, Pakistan	Low
Sesamia uniformis (Dudgeon)	Noctuidae	Rice, sorghum, Saccharum spp.	Northern India, Pakistan, the Philippines (?)	Low
Chilo orichalcociliellus (Strand)	Crambidae	Maize, sorghum, millet, sugarcane	Sub Saharan Africa	Low
Maliarpha separatella Ragonot**	Pyralidae	Rice	Africa, Indian Ocean islands, Burma and China (?)	Low
Chilo zacconius Bleszynski	Crambidae	Rice, sorghum, Echinochloa spp., cane	West Africa	Low
Diatraea guatemalella Schaus	Crambidae	Sugarcane	Mexico, Guatemala, Costa Rica	Low
Diatraea indigenella Dyar & Heinrich	Crambidae	Sugarcane	Colombia	Low
Diatraea magnifactella Dyar	Crambidae	Sugarcane	Mexico	Low
Sesamia nonagrioides Lefebvre	Noctuidae	Maize, rice, sorghum, sugarcane	Southern Europe to West Asia, West Africa to Sudan	Low
Sesamia poephaga Tams & Bowden	Noctuidae	Maize, sorghum, sugarcane	West Africa to Sudan, Comoros, Madagascar	Low
Diatraea dyari Box	Crambidae	Sugarcane	Argentina	Low
Diatraea tabernella Dyar	Crambidae	Sugarcane	Central America	Low
Diatraea veracruzana Box	Crambidae	Maize, sugarcane	Mexico	Low
Sesamia penniseti Tams & Bowden	Noctuidae	Rice, Saccharum spp., sorghum, maize	West Africa	Low

* Record from Northern Territory is now doubtful (ED Edwards, personal communication).
** Record from PNG by is now doubtful (L Kuniata, personal communication).
(?) Indicates uncertainty of record.

Table 2. Parasitoids, predators and pathogens recorded on sugarcane stem borers in Asia and Indian Ocean Islands.

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Parasitoids **Hymenoptera** **Bethylidae** *Goniozus* (*cuttackensis* Lal) *indicus* Ashmead	Ci[18], Cp[22,100], Cs[22], Ctum[22], Si[22], Sn[22]	L	Sugarcane, rice[100]	India, Philippines[100]	18, 21, 22, 100	
Goniozus indicus Ashmead	Cs	L	Sugarcane	India	18, 21	
Goniozus indicus Muesebeck	Cp	L	Rice[76]	India[76]	76	
Goniozus sp.	Ci,[18] Cp[36], Ed[14], Sn[22]	L	Sugarcane, maize[36]	Philippines[18], Taiwan[18], India[14,18,22], Pakistan[36]	14, 18, 21, 22, 27, 30, 36	
Braconidae *Agathis stigmatera* (Brullé)(*Alabagrus stigma* Cresson)	Cs	L	Sugarcane	Mauritius	52, 53, 54, 58	Introduced from Trinidad to Mauritius (1949-1951)[58]. Low parasitism levels recorded[54].
Allorhogas pyralophagus Marsh	As[126], Ca[126], Ci[126], Cp[146], Cs[52], Sn[144]	L	Sugarcane, sorghum[146]	India, Mauritius[52], Indonesia[144]	52, 126, 144, 146	Originally from Mexico. Introduced to India and recorded to have been established in release sites[126]. Introduced to Indonesia in 1982, long term impact unclear[144]. Introduced inro Mauritius but apparently unsuccessful[52]. A study in India failed to recover it from cane fields after release[47].
Apanteles sp.	Ca, Cs, Ctr[79,81], Ctum[22], Sc[64], Sn[18,117]	L	Sugarcane, maize[41,64]	Indonesia[117], India[41], Reunion[64], Philippines[18], PNG[79,81]	18, 22, 41, 64, 79, 81, 140, 117	
Apanteles sp. nr *chilonis* Munikata	Ctr	L	Sugarcane	PNG	79, 81	
Apanteles Baoris Wilkinson	Ca	L	Sugarcane	India	22	
Apanteles chilonis (Munakata)	Cp[123], Csup[62,63,70]	L	Rice[62,63,70]	India[123], Japan[62,63,70]	62, 63, 70, 123	
Apanteles (*Cotesia*) *flavipes* Cameron (*nonagriae* Olliff. nec Viereck, *Stenopleura simplicis* Viereck)	Su, As, Sn	L	Sugarcane	India	22	
Apanteles (*Cotesia*) *flavipes* Cam.	Se, Sn	L	Sugarcane	Philippines, Thailand	4, 135	
Apanteles flavipes Cam.	Cpc[69], Ctr[81]	L	Rice[69], sugarcane	Malaysia[69], PNG[81]	69, 81	
Apanteles flavipes Cam. (*A. nonagriae* Oll.)	Csup	L	Rice	Australia (NT)	77	
Apanteles flavipes Cameron	Su	L	Sugarcane	India, Philippines	18, 21	
Apanteles flavipes Cameron (*nonagriae* Ol. & Vier)	Sn	L	Sugarcane	India	21	
Apanteles pallipes Cameron	Si	L	Sugarcane	India	22	
Apanteles phytometrae Wilkinson	Ci	?	Sugarcane	India	22	
Apanteles ruficrus Hal.	Ca	L	Sugarcane, rice[154]	India, China[154]	101, 154	First record on this host in India[101].
Apanteles schoenobii Wilkinson	Cp	L	Sugarcane	India	22	
Apanteles scirpophagae Ashmead	Sn	L	Sugarcane	India	18, 22	
Ascogaster sp.	Ed	L	Sugarcane	India, Pakistan	21, 22	
Bracon albolineatus Cam.	Cp[71], Sc[94]	?	Sorghum[71], rice[94]	India[71], Mauritius[94]	71, 94	First record in India[71], introduced to Mauritius fot the control of *S. calamistis* but impact on pest unclear[94].
Bracon brevicornis Wesmael	Si	L	Sugarcane	India	22	

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Bracon chinensis (*Amyosoma, Microbracon*) (*albolineatus* Cameron, *chilonis* Viereck)	Ci, Si, Sn	L	Sugarcane	India, Taiwan, Philippines	18, 22	
Bracon chinensis Szepl.	Cs[58], Csup[69], Sc[58]	L	Sugarcane, rice[69]	Mauritius, Indonesia[69]	58, 69, 151	Introduced from Sri Lanka into Mauritius in 1939[58].
Bracon chinensis Szepligetti	Ci[18], Si[21], Su[18]	L	Sugarcane	India[18,21], Taiwan, Philippines	18, 21	
Bracon chinensis Szépligeti	Cp		Sugarcane, maize[24,99]	Pakistan[24], Nepal[99], Sri Lanka	21, 22, 24, 99	
Bracon famulus Bingham	Sn	L	Sugarcane	India	22	
Bracon hebetor Say	Si	L	Sugarcane	India	22	
Campyloneurus erythrothorax Szépl.	Cs	L	Sugarcane	Indonesia	69	
Campyloneurus mutator Fabricius	Ca, Ci, Ctum, Sn	L	Sugarcane	India	22	
Campyloneurus sp.	Ca, Cs	L	Sugarcane	Indonesia	116, 140	
Chelonus heliopae Gupta	Cp	L	Sugarcane	India	22	
Chelonus narayani Subba Rao	Ed	?	Sugarcane	India	22	
Chelonus munakatae	Ci	L	Millet	China	80	
Chilonis sp.	Sn	L	Sugarcane	India	18, 21, 22	
Chelonus sp. (b)	Cp, Ed	L	Sugarcane	India, Pakistan	21, 22	
Cotesia (*Apanteles*) *flavipes*	Ci	L	Sugacane, Vetiver grass (*Vetiveria zizamoides*)	India[18,21,22,98,133], Pakistan[90], Philippines[18], Taiwan[29], Thailand[135]	18, 21, 22, 29, 133, 90, 98, 135	A number of *C. flavipes* sugarcane adapted strains were imported from Indonesia, Thailand and Barbados, bred freely among themselves and released in Pakistan in 1983 and in the Punjab in 1982-1985. This resulted in successful establishment in sugarcane[91].
Cotesia (*Apanteles*) *flavipes* Cameron	Cp[2,18,19,71,93,97,98,99,128,133,136], Cs[8,13,52,53,54,58,69,89,94,109,135,151,152], Si[1,10,18,24,29,72,88,98,115]	L	Maize[2,19,93,99,128,136], sorghum[2,71,98,133], sugarcane, rice[98,115], cattail (*Typha angustata*)[24], *Saccharum spontaneum*[98], *Erianthus arundinaceus*[98], Job's tears (*Coix lachrymal-jobi*) L[97].	India[18,21,22,44,47,72,98,133,136], Pakistan[2,42,90,93], Nepal[99], Comoros[19], Sri Lanka[18], Taiwan[18,29], Mauritius [52,53,54,58,94,109,151,152], Madagascar[8,13], Reunion[58], Thailand[135], Indonesia[69,89,138], Japan[1,10], Philippines[18]	1, 2, 8, 10, 13, 18, 19, 24, 29, 44, 47, 58, 71, 72, 88, 89, 93, 97, 98, 99, 115, 133, 135, 136	• It is suggested that a shipment of *Apanteles* sp. (possibly *Cotesia flavipes*) arrived in Mauritius from India in 1964[58]. Another theory suggests *Cotesia flavipes* was introduced into Mauritius in 1917, and later into the Reunion[7]. It is also possible that *C. flavipes* may have arrived with it's host around 1850 from India[58]. • Strain in Madagascar was originally introduced from Mauritius in 1960 – 1961, well established[8]. • A Japanese strain was introduced into Pakistan in 1962, well established[2]. • A hybrid between a sugarcane-adapted strain, from Indonesia, and a local maize-adapted strain did establish in sugarcane in the Sindh Province of Pakistan[90]. • An imported Thai strain in 1985 improved overall parasitism rates on both hosts in Indonesia[89].

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Cotesia flavipes Cameron	As [91,96,124], Args[29], Ca[22,97,98,101], Csup[29,70], Ctum[16,17,134], Sg[74,75]	L	Sugarcane, *Sacciolepis interrupta*[98*], rice[29]	India [16,17,22,91,97,98,101,124], Pakistan[96], Indonesia[91,116,138], Japan, Taiwan[29], Thailand[134], PNG[74,75]	16,17,22,29,70,74,75,91,96,97,98,101,116,124,138	• *C. auricilius* larvae in Indonesia used to encapsulate immatures of the Indonesian *C. flavipes*. A Thai strain was introduced to Indonesia in 1985 that resulted in high parasitism rates[91]. • Record of *C. suppressalis* in cane (ref 29) is probably a misidentification, or pest was found occasionally in cane. • An indigenous population in PNG is responsible for high levels of parasitism (up to 70%). Continuously mass released[74,75].
Cotesia (*Apanteles*) *sesamiae*	Sc	L	Sugarcane, maize [19,58], sorghum [12,1]	Mauritius, Madagascar, Reunion	6, 12, 19, 52, 53, 58, 109, 151	Originally from East Africa, *C. sesamiae* was introduced into Mauritius in 1951 from Kenya, and later from Mauritius into the Reunion in 1953-1955. Well established [6,58]. It was also introduced from Uganda to Madagascar [19], well established.
Glyptomorpha (=*Stenobracon*) *nicevillei* Bingham	Se	L	Sugarcane	India	142	
Habrobracon hebetor L.	Scrt	L	Maize	Iran	127	
Hormiopterus (*Rhaconotus*) sp.	Cs	L	Sugarcane	Indonesia	69	
Iphiaulax famulus Bingham	Si, Su, Sn	L	Sugarcane	India, Philippines	18,22	
Iphiaulax sikkimenis Cameron	Sn	L	Sugarcane	India	22	
Iphiaulax sp.	Si, Sn	L	Sugarcane	India	22	
Iphiaulax spilocephalus Cameron	Cp	L	Sugarcane	India	21,22	
Macrocentrus jacobsoni Szépl.	Ci, Cs, Sn	L	Sugarcane	Taiwan	18	
Macrocentrus nicevillei Ashmead	Si	L	Sugarcane	India	22	
Merinotus sp.	Cp	?	Sugarcane	India	22	
Microbracon chilocida Ram.	Cp	?	Sugarcane	India	22	
Microbracon chinensis	Ci, Cs	L	Sugarcane	Taiwan	29,34	
Microplitis sp.	Cp	?	Sugarcane	India	22	
Phanerotoma hendecasiella Cam.	Ed	?	Sugarcane	India	18	
Pseudoshirakia sp.	Sc	L	Sugarcane	India	42,142	
Rhaconotus roslinesis Lal	Sn	L	Sugarcane	India	21	
Rhaconotus roslinensis Lal (=*caulicola* Muesebeck)	Cs, As	L	Sugarcane	India	21,22	
Rhaconotus schoenobii Roh.	Sn	?	Sugarcane	Philippines	18	

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Rhaconotus scirpophagae Wilkinson	As[18,21,22], Ed[18], Sn[18,21,22,24,57], Cp[21,22], Se[60,95,142]	L	Sugarcane	India, Pakistan[24]	18, 21, 22, 24, 57, 60, 95, 142	Recorded as the most common larval parasitoid on this host in Pakistan[24]. Parasitism levels of up to 33.42% were recorded in North Bihar, India[60].
Rhaconotus signipennis Walker	As, Cs, Sn	L	Sugarcane	India	22,125	
Rhaconotus sp.	Se	L	Sugarcane	India	103	
Shirakia schoenobii Vier	Si	L	Sugarcane	Taiwan	18	
Shirakia sp.	Sn	?	Sugarcane	India	21	
Shirakia yokohamensis Cam.	Sn	L	Sugarcane	Taiwan	18	
Spathius elaboratus Wilkinson	As	L	Sugarcane	India	121	New record.
Spathius sp.	Se	L	Sugarcane	India	142	
Stenobracon (*Bracon, Glyptomorpha*) *karnalensis* Lal	Sn	L	Sugarcane	India	21	
Stenobracon deesae Cameron	As, Ca, Ci[21,22,24], Cp[21,22,24], Cs[47], Ed[18], Se[60,95], Sn[21,24]	L, P(?)[95] [*]	Sugarcane, maize[24]	India, Pakistan[24]	18, 20, 21, 24, 47, 60, 95, 142	Low parasitism levels recorded in Pakistan on *C. infuscatellus* (5.1%)[24] and on *S. nivella* (<3.1%)[24], while higher levels were recorded in North Bihar, India (up to 54.23%)[60].
Stenobracon karnalensis Lal	Sn	L	Sugarcane	India	22	
Stenobracon nicevillei Bingham	As[22], Ci[22], Cp[20,21,22,99], Sn[21,22,57]	L	Sugarcane, (rice, maize & sorghum)[99]	India, Nepal[99]	20,21,22,57,99	Possibly a synonym of *S. maculata* Vier., a rice stem borer parasitoid in Taiwan.
Stenobracon sp.	Sn	L	Sugarcane	Indonesia	140	
Stenobracon trifasciatus Szépl.	Ci, Sn	L	Sugarcane	Taiwan, Indonesia	18,69,117	
Tropobracon (*Shirakia*) *schoenobii* (Viereck)	Ca, Ci, Cp, Si	?	Sugarcane, rice	India	22	
Vipio sp.	Ca, Cp, Si, Sn	L	Sugarcane	India	22	
Vipio (*Stenobracon, Bracon, Glyptomorpha*) *deesae* (Cameron)	As, Ca, Ci, Cp, Ed, Sn	L	Sugarcane	India	22	
Ceraphronidae						
Ceraphron (*Calliceras*) *fijiensis* Ferriere	Si	?	Sugarcane	India	22	Possibly a hyperparasitoid on *Cotesia flavipes* (see Chaudhary & Chand 1972).
Ceraphron sp.	Ctr	L	Sugarcane	PNG	81	

[*] *Stenobracon deesae* Cam. is a larval parasitoid, this record could be a misidentification or possibly an error.

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Chalcididae						
Bephratoides saccharicola Mani	Sc	?	Sugarcane	India	21, 22	
Brachymeria (*Chalcis*) sp.	Si	P	Sugarcane	India	22	
Euchalcidia sp.	Cpc	P	Rice	Australia (NT)	77	
Harmoniae sp.	Sc	L	Sugarcane	India, Pakistan	21, 22	
Hyperchalcidia soudanensis Steffan	Cp	P	Rice, maize & sorghum	Nepal	99	
Hyperchalcidia sp.	Cp	P	Maize	Pakistan	24	
Neohybothorax sp.	Ed	?	Sugarcane	India	118	New record in India.
Trichospilus diatraea Chairman & Margabandhu	Cs	P	Sugarcane	India, Mauritius	18, 21, 22, 52, 53, 58, 151	Introduced into Mauritius from India in 1959, established[58].
Elasmidae *Elasmus* sp.	Sn	L	Sugarcane	Taiwan, Indonesia	18, 140	
Elasmus zehntneri Ferr.	Se	L	Sugarcane	India, Indonesia, Pakistan, Philippines	18, 21, 22, 24, 60, 69, 103, 141, 142, 145, 117	Mass released in Indonesia[145]. Low parasitism levels recorded in India and Pakistan [24,60].
Eucoilidae *Rhoptromeris* sp.	Se	L	Sugarcane	India	103	
Eulophidae *Anostocetus* sp.	Ctum, Sn	L	Sugarcane	India	21, 22	
Aprostocetus sp.	Ci, Cp, Sn	P	Sugarcane	India	21, 22	
Pediobius furvus (Gahan)	Cp, Sc [7,12,58,93], Sg[73,75,85]	P	Sugarcane, maize[19], rice[7], sorghum[12]	Comoros[19], Madagascar[12], Reunion[12], PNG[73,75,85]	7, 12, 19, 58, 73, 75, 85, 93	Introduced into Madagascar and the Reunion from Uganda in 1968 - 1971, well established[12]. Later introduced from Madagascar to Comoros in 1969-1971, established[19]. Also introduced from Kenya into PNG, established, but parasitism levels are generally low[75].
Tetrastichus atriclavus Waterst	Cs	P	Sugarcane	Mauritius	52, 54	Introduced into Mauritius, low parasitism levels recorded[54].
Tetrastichus ayyari Rohwer	Ci, Cp[12], Cs, Si, Sn	P	Sugarcane, sorghum[12]	India, Reunion[12]	12, 21, 22	
Tetrastichus israeli (M.&K.)	Csup[69], Sc[12], Si[69]	P	Rice[69], sorghum[12]	Indonesia[69], Reunion[12]	12, 69	Introduced from India into Reunion in 1959[12].
Tetrastichus israeli Mani & Kurian (*Aprostocetus israeli* Mani)	Ca, Ci	P	Sugarcane, rice	India	22	
Tetrastichus schoenobii Ferriere	Ci, Sn[89]	E	Sugarcane	India, Indonesia [18, 89]	18, 22, 89	
Tetrastichus scirpophaga Mani	Sn	E	Sugarcane	India	22	
Tetrastichus sp.	Ci, Cs, Sn	P	Sugarcane	India, Indonesia	21, 22, 44, 140	
Tetrastichus sp. (near *atriclavus* Waterst.)	Cs, Sc	P	Sugarcane	Mauritius	18, 54, 94	
Trichospilus diatraea Chairman & Margabandhu	Sc[12], Si[22]	P	Sugarcane, sorghum[12]	India, Reunion[12], Mauritius	12, 22, 151	Introduced from India into Mauritius in 1963-1964[151].

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Eupelmidae						
Eupelmus sp.	Ca	L?	Rice	India	22	
Ichneumonidae *Amauromorpha metathoracio schoenobii* Viereck	Ca, Cs[18]	L	Sugarcane	India, Indonesia[18]	18,22	
Amauromorpha schoenobii Vier.	Si, Sn	?	Sugarcane	Taiwan	18	
Anomalon sp.	Sn	L	Sugarcane	India	22	
Brachycoryphus nersei Cameron	Ci	L, P	Sugarcane	India	22	
Centeterus alternecoloratus Cushman	Ca[22,25], Ci, Cp[25], Sn	P	Sugarcane, rice[25], maize [22,25]	India	22,25	Recorded as a key pupal parasitoids in India with up to 50% parasitism levels[25]. 33% parasitism levels recorded in Assam, India[25].
Cremastus sp.	As, Sn	L	Sugarcane	India	21,22	
Cremastus (*Trathala*) *flavo-orbitalis* (Cameron)	Ca, Cp	L	Rice[22], sugarcane	India, Sri Lanka	18,22	
Enicospilus antankarus Sauss.	Cs	?	Sugarcane	Mauritius	18	
Enicosphilus (*Enicospilus*) *terebrus* Gauld	Sg	L	Sugarcane	PNG	74,75	Low parasitism levels recorded in PNG[75].
Enicospilus sakaguchii Mats. & Uchida	Si	?	Sugarcane	Taiwan	18	
Enicospilus sp.	Sc	L	Sugarcane	Mauritius	18,94	
Exetastes longicornis Ishida	Sn	?	Sugarcane	Taiwan	18	
Gambroides dammermani Rohw.	Sn	?	Sugarcane	Philippines	18	
Gambroides javensis Rohw.	Sn	?	Sugarcane	Indonesia, Philippines	18	
Gambroides rufithorax Uchida	Cs	?	Sugarcane	Taiwan	18	
Gambroides sp.	Ca, Cs	P	Sugarcane	Indonesia	140	
Goryphus basilaris Holmgren (*Exetastes, Mesosternus longicornis* Ishida)	Cs, Sn	?	Sugarcane	India	22	
Goryphus (*Melcha*) *ornatipennis* Cameron	Cs	?	Sugarcane	India	22	
Goryphus sp.	Cs, Sn	L?	Sugarcane	India	21,22	
Gotra marginata Brulle (*Listrognathus marginatus* WLK)	Ci	L?	Sugarcane	India	21,22	
Habropimpla sesamiae Rao	Sc	P	Sugarcane	India	21	
Horogenes lineata Ishida	Ci, Si	?	Sugarcane	India, Taiwan	18,21	
Ichneumon unicinctus Brúlle	Sc	P?	Sugarcane	Mauritius	151	
Ischnojoppa luteator Fab.	Sn	P	Sugarcane	India	22	
Isotima javensis Rhower	Se[47,60,95,103,142], Sn[57,69,106,117]	L[57], P[69], PP[106]	Sugarcane	India, Indonesia[69,117]	47,57,60, 69,95, 103, 106,117,142	A key parasitoid of *S. nivella* in India[106]. Parasitism levels of 6.67 – 15.28% were recorded in India on *S. excerptalis*[60].
Isotima dammermani Rohwer	Sn	P	Sugarcane	India	22	
Isotima (*Melcha, Gambroides, Eripernimorpha*) *javensis* Rohwer	Sn	?	Sugarcane	India	22	

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Isotima sp.	Ci[3,24], Sn[24]	L	Sugarcane	Pakistan[24], Philippines	3,24	Low parasitism levels recorded in Pakistan[24].
Kriegeria heptazonata Ashm.	Su, Sn	?	Sugarcane	Philippines	18	
Kriegeria sp.	Sn	?	Sugarcane	India	22	
Listrognathus (*Mesostenoideus*) *calvinervis* Cameron	Sn	L	Sugarcane	India	21	
Melcha ornatipennis Cameron	Ci, Sn[18]	P	Sugarcane	India, Burma[18]	18,21,22	
Meloboris sinicus (Holmgren)	Ci, Cs	L	Sugarcane	Taiwan	29,30,33	
Mesostenus longicornis Ishida	Ci, Sn	?	Sugarcane	India	18	
Metopius sesamiae Rao	Si	P	Sugarcane	India	21	
Pimpla predator Fabricius	Sn	P	Sugarcane	India	18	
Syzeuctus sp.	Sn	L	Sugarcane	India	22	
Temelucha philippinensis (Ashmead)	Se	L	Sugarcane	Thailand	135	
Temelucha sp.	Si[22], Se[103,124], Sn[22]	L	Sugarcane, rice[22]	India	22,103,142	
Trathala flavoorbitalis Cameron	Cp	L	(Rice, maize & sorghum)[99]	Nepal	99	
Vulgichneumon leucaniae Uchida	Si	P	?	China	78	
Xanthopimpla citrina (*X. luteola*) (Hlmgr.)	Cs[18,52,94], Sc[18,58,94]	P	Sugarcane	Mauritius[18,52,94], Reunion[58]	18,52,58,94	Introduced from Sri Lanka into Mauritius in 1952-1953, and in 1953, 1960 from Mauritius to Reunion[58,94].
Xanthopimpla enderleini Krieg.	Si, Su	?	Sugarcane	Philippines	18	
Xanthopimpla (*Metopis*) *sesamiae* (Rao)	Si	?	Sugarcane	India	22	
Xanthopimpla nursei Cameron	Cp	P	Sugarcane	India	21	
Xanthopimpla pedator F.	Se	P	Sugarcane	India	95	
Xanthopimpla (*Pimpla*) *punctata* Fabricus	Ci	P	Sugarcane	India	22	
Xanthopimpla predator Fabricius	Cp	P	Sugarcane	India	21	
Xanthopimpla punctator (*predator* Fabricius) Linnaeus	Cp	P	Sugarcane	India	22	
Xanthopimpla sp.	Ca, Cs, Ctum[134]	P	Sugarcane	Indonesia, Thailand[134]	134,140	
Xanthopimpla stemmator Thunberg	Ca[116], Ci[30, 132], Cp [18,99], Cs[58], Csup[69], Sc [58,94], Si [18, 132], Sn[139]	P	Sugarcane, (rice, maize & sorghum)[99]	India, Sri Lanka[18], Nepal[99], Indonesia[69], Taiwan[30,132,139], Mauritius[58], Reunion[23]	18,23,30,52,53,54, 58,69,94,	Introduced from Sri Lanka into Mauritius in (1939-1942) and few individuals released, well established[58]. Later in 1953, 1966 it was introduced from Mauritius into Reunion[58], well established.
Xanthopimpla stemmator Thunberg (*thoracalis* Krieger, *bimaculata* Cameron *maculifrons* Cameron, *nursei* Cameron, *fascialis* Szepligetti, *Habropimpla sesamiae* Rao)	Ci, Cp, Cs, Sn	P	Sugarcane	India	22	

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Xanthopimpla stemmator Timberlake	Cp	P	Sugarcane, (rice, maize & sorghum)[99]	India, Pakistan[24], Sri Lanka[147], Taiwan[18]	18,21,24,99,147[*]	
Pteromalidae *Dinarmus* sp	Sn	L?	Sugarcane	Indonesia	69	
Scelionidae *Gryon nixoni* Masner	Ctr	E	Sugarcane	PNG	81	
Platytelenomus busseolae Gahan	Scrt	E	Maize	Iran	127	
Platytelenomus sp. (? *hylas* Nixon)	Sc	E	Sugarcane	Mauritius	94	
Telenomus alecto Crawford	Ci	E	Sugarcane	India	22	Introduced from Colombia, well established in West Bengal.
Telenomus beneficiens Nixon	Cs	E	Sugarcane	India	21,22,45,110,111	
Telenomus beneficiens var. *elongatus* Ishida	Sn	E	Sugarcane	Taiwan	18, 35	The key egg parasitoid in cane fields in Taiwan[35].
Telenomus beneficiens (Zehntner)	Sn, Cs[28], Ci[28]	E	Sugarcane	Taiwan[28], Indonesia[18,69,117], India[18], Philippines[18]	18,28, 69,117	
Telenomus beneficiens (Zehnt.) Nixon	Cs	E	Sugarcane	India	44	
Telenomus beneficiens (Zehntner) (Ceraphron)	Cs	E	Sugarcane	Mauritius[18], Taiwan[18], Indonesia[18], China[32]	18, 32	
Telenomus (*Ceraphron, Phanurus, Praphanurus*) *beneficiens* (Zehntner) Nixon	Ci, Sn	E	Sugarcane	India	21	
Telenomus dignoides Nixon	Ci, Cs, Se[4], Sn[21,22, 24,89]	E	Sugarcane	Indonesia[89], Pakistan[24], India, Philippines[4]	4, 21, 22, 24, 44, 45, 89	
Telenomus dignus Gah.	Csup[69], Se[4, 103,142], Sn[21,22]	E	Sugarcane, rice[69]	Indonesia, India, Philippines	4, 21,22, 69, 103, 142	
Telenomus globosus n. sp.	Cs	E	Sugarcane	India	15, 44	
Telenomus (*Phanurus, Praphanurus*) *beneficiens* (Zehntner) (Ceraphron)	Ci, Sn	E	Sugarcane	India	22	
Telenomus rowani (Gahan)	Ci, Cs, Ctum[134], Se, Sn[21,22]	E	Sugarcane	Thailand[134], India[21,22]	21, 22, 134, 135	
Telenomus saccharicola Mani	Sn	E	Sugarcane	India	22	
Telenomus sp.	Ca, Ci, Cp[69], Cs[69,140], Ctr[81, 153], Sg[74,75], Si[21], Sn[22,57,140]	E	Sugarcane, rice[69]	Indonesia[140], India[21,22,57], Malaysia[69,140], PNG[81,153]	21, 22, 69, 74, 75, 81, 153, 140	An indigenous strain is used for augmentative releases in PNG[74,75].
Trichogrammatidae *Trichogramma australicum* Girault	Ci [18,22,61], Cs[18,52,53,54,58], Sc[18, 58], Ed[22]	E	Sugarcane	India[22], Indonesia[18], Taiwan[18], Pakistan[61], Mauritius[18,52,53,54,58]	18, 22, 52, 53, 54, 58, 61	Introduced from India into Mauritius in 1964, well established[58].
Trichogramma bactrea Nagaraja	Ci[40], Cs[40]	E	Sugarcane	India[40]	40	

[*] Apparently a misidentification of the host (*C. partellus*) (See Greathead 1971).

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Trichogramma chilonis Ishii	Ca[89,129], Ci [4,9,40,66,84,90,91,143], Cp [37,40,99,143], Cs[27,30,56,44,45,111,112,113,114,122], Si[84], As[143], Ed[9], Se[103,142], Args[4,29]	E	Sugarcane, Sorghum[37,99], Rice & Maize[99].	India [37,40,44,45,103,111,112,113,114,120,122,129,142,143], Indonesia[91,89], Taiwan[27,29,30], China[84], Pakistan[9,90], Philippines[4,66], Nepal[99], Reunion[56]	4, 9, 29, 30, 37,40, 44, 45, 56, 66, 84, 89, 90, 91, 99, 103, 111, 112, 113, 114, 120, 122, 129, 142, 143	A strain from Taiwan is mass released in India[143]. Mass released in Indonesia[89] and Pakistan[9,90]. Widely mass released in India[40,111,129 3'] and the Punjab[143]. Augmentative early releases early in the season increased parasitism rates to almost 98% in Indonesia[91].
Trichogramma chilotraeae Nagaraja and Nagarkatti	Ci[4,40,86,135], Cs[135], Ctum[134], Se[135], Args[4]	E	Sugarcane	Philippines[4], Thailand[86,134,135], India[40]	4, 40, 86, 134,135	
Trichogramma confusum (*T. chilonis*)	Ci[38,82], Cs[38,82], Args[148]	E	Sugarcane	China[38,82,148]	38,82, 148	Mass released in China[38,82].
Trichogramma dendrolimi	Args	E	Sugarcane	China	148	
Trichogramma exiguum	Cp[67]	E	Sorghum[37,67]	India [37,67]	37,67	Different strains were introduced from Barbados, Colombia and the Philippines, well established in Delhi and Nagpur[37].
Trichogramma evanescens minutum Riley	Ci ,Cp[21], Cs[21], Su[21]	E	Sugarcane	India	21	
Trichogramma fasciatum (Perkins)	Se	E	Sugarcane	India	104	Introduced from Barbados.
Trichogramma flandersi Nagaraja & Nagarkatti	Ci	E	Sugarcane	India	40	
Trichogramma japonicum Ashmead	Ca[18], Ci[22], Se[103], Args[148]	E	Sugarcane	India[22,103], Taiwan[18], China[148]	18, 22,103, 148	Mass released in India[103].
Trichogramma minutum Riley	Ci[18], Ed[18]	E	Sugarcane	India[18]	18	
Trichogramma nagarkattii	Ci[59]	E	Sugarcane	China[59]	59	Mass released in China[59].
Trichogramma nanum Zhnt.	Ca[18], Ci [4,18], Cs[18]	E	Sugarcane	Malaysia[18], India[18], Indonesia[18], Philippines[4], Taiwan[18]	4, 18	
Trichogramma nr. *nana* (Zhnt.)	Cs[18,69]	E	Sugarcane	Indonesia[69], Madagascar[18], Taiwan[18]	18, 69	
Trichogramma nubilale Ertle & Davis	Ci[59], Cs[83], Args[83]	E	Sugarcane	China[59,83]	59, 83	Introduced from USA into China in 1983[83]. Mass released [59,83].
Trichogramma ostriniae	Args	E	Sugarcane	Taiwan[31], China[148]	31, 148	
Trichogramma plasseyensis Nagaraja	Ci	E	Sugarcane	India	40	
Trichogramma poliae Nagaraja	Ci	E	Sugarcane	India	40	
Trichogramma semblidis (Auriv.)	Ci	E	Sugarcane	India	40	
Trichogramma sp.	Se[5], Args[5,52]	E	Sugarcane	Mauritius[52], Philippines[5]	5	
Trichogramma sp. (near *nana* (Zehnt))	Sc, Args[94,151]	E	Sugarcane	Mauritius[94,151], Madagascar[18]	18, 94,151	
Trichogramma sp. nr. *plasseyensis* Nagaraja	Ctr	E	Sugarcane	PNG	81	
Trichogramma spp.	Ca[140], Ci[5], Cpc[69], Csup[69], Ctr[79,153]	E	Sugarcane, rice[69]	Philippines[5], Indonesia [69,140], Malaysia[69], PNG[79,153]	5, 69, 79, 140, 153	
Trichogramma spp. (? *australicum* Girault)	Cs[94], Sc[94], Args[151]	E	Sugarcane	Mauritius[94,151]	94, 151	
Trichogrammatoidea nana Zehnt.	Args	E	Sugarcane	Indonesia[102], Philippines[4]	4, 102	Mass released in Indonesia[102]. The main egg parasitoid in the Philippines, 91% parasitism rates recorded[4].
Trichogrammatoidea nana Zehntner	Ci	E	Sugarcane	India	21,22	

' David and Easwaramoorthy (1990) state that *T. chilonis* was formerly misidentified in India as *Trichogramma evanescens minutum*, *Trichogramma australicum* and *Trichogramma confusum*.

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Diptera						
Chloropidae						
Anacamptoneurum oblicunum Becker	Si	?	Sugarcane	India	22	
Anacamptoneurum sp.	Si	?	Sugarcane	India	22	
Anatrichus erinaceous Loew	Si	?	Sugarcane	India	22	
Mepachymerus (*Stellocerus*) *tenellus* Becker	Ci	?	Sugarcane	India	22	
Mepachymerus (*Stellocerus*) *tenellus* Becker	Si	?	Sugarcane	India	22	
Empididae *Drapetis* sp.	Ci	L	Sugarcane	India	22	
Phoridae	Cp	?	Sugarcane	India	22	
Tachinidae *Carcelia* sp.	Cs[69], Sg[75,85]	L	Sugarcane	Indonesia, PNG[75,85]	69, 75, 85	Low levels of parasitism recorded in PNG[75].
Carcelia (*Senametopia*) sp.	Ctr	L	Sugarcane	PNG	81	
Diatraeophaga sp.	Cs	P	Sugarcane	Indonesia[69]	69	Mass released in Indonesia[69]
Diatraeophaga striatalis Tns.	Ca[116], Cs[18,40]	L, P[18]	Sugarcane	Indonesia [18,116], India[40]	18, 40, 116	Mass released in Indonesia[116]. Imported from java and released in Tamil Nadu, India, in 1979, later recovered from release sites[40].
Dichaetomyia pallitarsus (Stein)	Cpc	P	Rice	Malaysia	69	
Drino discreta Van der Wulp	Si	?	Sugarcane	India	22	
Exorista quadrimaculata Baranov	Ci	L	Sugarcane	India	22	
Lixophaga diatrae (*diatraeae*)	Ci	L	Sugarcane	Philippines[4]	4	Introduced to the Philippines from South America, resulted in low parasitism levels[4].
Pseudoperichaeta orientalis Wiedmann	Si	L	Sugarcane	India	22	
Schistochilus aristatum Aldr.	Cs	?	Sugarcane	Indonesia	18	
Sturmiopsis inferens Townsend	Ca, Ci, Cp, Cs, Csup[69], Cpc [69], Si[22], As[39], Sn[22]	L	Sugarcane, rice[69]	India, Malaysia [69], Indonesia [89]	22, 26, 39, 51, 65, 69, 89, 106, 108	Mass released in Indonesia[89].
Sturmiopsis (*Winthemia*) *semiberbis* Bezzi	Ci, Cp, Si	L	Sugarcane	India	21	
Predators						
Anisolabiidae *Euborellia stali* Dohn.	Si	L	Rice	Philippines	11	
Anthocoridae *Blaptostethoides* sp.	Sg	E	Sugarcane	PNG	75	
Chelisochidae *Chelisoches morio* (F.)	Sg	E, L	Sugarcane	PNG	75	
Carabidae						
Hexagonia sp? *Insignis* (Bates)	Cs	E, (L?)	Sugarcane	India	44	

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Chrysopidae *Chrysopa* sp.	Cs	E	Sugarcane	Indonesia	69	
Coccinellidae *Brumus* (*Coccinella*) *suturalis* Fabricius	Sn	E	Sugarcane	India	22	
Brumus suturalis F.	Sn	E	Sugarcane	India	21	
Menochilus sexmaculatus (Fabricius)	CP	L	Sorghum	India	68	
Forficulidae						
Forficula sp.	Ca	L	Sugarcane	India	22	
Formicidae *Anoplolepis longipes* Jerdon	Cs	E, (L?)	Sugarcane	India	44	
Camponotus compressus (F.)	Cs	E, (L?)	Sugarcane	India	44	
Camponotus rufogloucus (Jerdon)	Cs	E, (L?)	Sugarcane	India	44	
Irridomymex spp.	Sg	L, P	Sugarcane	PNG	44	
Monomorium aberrans Forel	Cs	E, (L?)	Sugarcane	India	44	
Monomorium sp.	Sn	L, P	Sugarcane	India	22	
Oecophylla amaragdina Fabr.	Cs	E, (L?)	Sugarcane	India	44	
Pheidole megacephala Fab.	Cs, Args[151]	E	Sugarcane	Reunion[56], Mauritius[151]	56, 151	
Pheidole sp.	Sg	L, P	Sugarcane	PNG	75	
Pheldiogeton sp.	Cs	E, (L?)	Sugarcane	India	44	
Solinopsis geminala (F.)	Cs	E, (L?)	Sugarcane	India	44	
Tetraponera refonigra Jerdon	Cs	E, (L?)	Sugarcane	India	44	
Glubionidae *Oedignatha* sp.	Cs	E, (L?)	Sugarcane	India	44	
Lycosidae *Hippasa greenalliae* (Blackwell)	Ci[49]	L	Sugarcane	India	49	
Paradosa sp.	Cs	E, (L?)	Sugarcane	India	44	
Oxyopidae						
Oxyopes sp.	Cs	E, (L?)	Sugarcane	India	44	
Pentatomidae *Amyotea* (*asopus*) *malabarica* (Fabricius)	Si	L	Rice	India	105	
Reduviidae						
Acanthaspis quinquespinosa Fabricius	Cp	L	Sugarcane	India	21, 22	
Salticidae *Carrhotus viduus* Koch	Cs	E, (L?)	Sugarcane	India	44	
Plexippus paykulli (Audouin)	Cs	E, (L?)	Sugarcane	India	44	
Staphylinidae *Paederus fucipes* Curtis	Cp	E	Maize	Pakistan	92	
Thomisidae *Runcinia* sp.	Cs	E, (L?)	Sugarcane	India	44	

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Chrysopidae *Chrysopa* sp.	Cs	E	Sugarcane	Indonesia	69	
Coccinellidae *Brumus (Coccinella) suturalis* Fabricius	Sn	E	Sugarcane	India	22	
Brumus suturalis F.	Sn	E	Sugarcane	India	21	
Menochilus sexmaculatus (Fabricius)	CP	L	Sorghum	India	68	
Forficulidae						
Forficula sp.	Ca	L	Sugarcane	India	22	
Formicidae *Anoplolepis longipes* Jerdon	Cs	E, (L?)	Sugarcane	India	44	
Camponotus compressus (F.)	Cs	E, (L?)	Sugarcane	India	44	
Camponotus rufogloucus (Jerdon)	Cs	E, (L?)	Sugarcane	India	44	
Irridomymex spp.	Sg	L, P	Sugarcane	PNG	44	
Monomorium aberrans Forel	Cs	E, (L?)	Sugarcane	India	44	
Monomorium sp.	Sn	L, P	Sugarcane	India	22	
Oecophylla amaragdina Fabr.	Cs	E, (L?)	Sugarcane	India	44	
Pheidole megacephala Fab.	Cs, Args[151]	E	Sugarcane	Reunion[56], Mauritius[151]	56, 151	
Pheidole sp.	Sg	L, P	Sugarcane	PNG	75	
Pheldiogeton sp.	Cs	E, (L?)	Sugarcane	India	44	
Solinopsis geminala (F.)	Cs	E, (L?)	Sugarcane	India	44	
Tetraponera refonigra Jerdon	Cs	E, (L?)	Sugarcane	India	44	
Glubionidae *Oedignatha* sp.	Cs	E, (L?)	Sugarcane	India	44	
Lycosidae *Hippasa greenalliae* (Blackwell)	Ci[49]	L	Sugarcane	India	49	
Paradosa sp.	Cs	E, (L?)	Sugarcane	India	44	
Oxyopidae						
Oxyopes sp.	Cs	E, (L?)	Sugarcane	India	44	
Pentatomidae *Amyotea (asopus) malabarica* (Fabricius)	Si	L	Rice	India	105	
Reduviidae						
Acanthaspis quinquespinosa Fabricius	Cp	L	Sugarcane	India	21, 22	
Salticidae *Carrhotus viduus* Koch	Cs	E, (L?)	Sugarcane	India	44	
Plexippus paykulli (Audouin)	Cs	E, (L?)	Sugarcane	India	44	
Staphylinidae *Paederus fucipes* Curtis	Cp	E	Maize	Pakistan	92	

Table 2. (*Continued*)

	Host attacked	Stage attacked	Host plant	Country	Reference	Remarks
Pathogens						
Bacillaceae *Bacillus thuringiensis* Berliner	Cp	L	Sorghum	India	137	
Heterorhabditidae *Heterorhabditis indicus* n. sp.	Se	L	Sugarcane	India	107	
Hypomycetes						
Beauveria bassiana	Sg, Ed[119]	L	Sugarcane	PNG, India[119]	73, 119	
Beauveria densa	Cp	L	Sorghum	India	137	
Hirsutella nodulosa Petch	Cs	L	Sugarcane	India	48, 50	
Beauveria nr. *bassiana*	Ci	L	Sugarcane	India	130	
Metarhizium anisopliae (Metschnikoff)	Cs[53], Sg[75], Ed[119]	L, P[75]	Sugarcane	Mauritius[53], PNG[75], India[119]	53, 75, 119	
Paecilomyces sp.	Cs	L	Sugarcane	Mauritius	53	
Mermithidae						
Amphimermis sp.	Ci	L	Sugarcane	Pakistan	24	
Hexamermis sp.	Cp	L	Sorghum	India	137	
Mermis sp.	Cs, Sc	L	Sugarcane	Mauritius	94	
Nosematidae						
Nosema furnacalis	Cs	?	?	China	149	
Nosema infuscatellus	Ci	L	Sugarcane	China	150	
Nosema sp.	Cp	L	Sorghum	India	46	
Protozoa						
Tetrahymena sp.	Cp	L	Sorghum	India	137	
Rhabditida						
Rhabditis sp.	Cp	L	Sorghum	India	137	
Panagrolaimus sp.	Cp	L	Sorghum	India	137	
Steinernematidae						
Neoaplectana sp.	Cp	L	Sorghum	India	137	
Viruses						
Cytoplasmic polyhedral virus	Sc	L	Maize, cane	Reunion	64	
Granulosis virus (GV)	Ci, Cs	L	Sugarcane	India	43, 44, 87	
Nuclear polyhedral virus	Sc	L	Maize, cane	Reunion	64	
Nuclear polyhedrosis virus	Si	L	Rice	India[55], Korea[131]	55, 131	

Args=*Argyroploce* (*Tetramoera*) *schistaceana*; As= *Acigona steniellus*; Ca=*Chilo auricilius*, Ci=*Chilo infuscatellus*; Cp=*Chilo partellus*; Cpc=*Chilo polychrysus*; Cs=*Chilo sacchariphagus*; Csup=*Chilo suppressalis*; Ctr=*Chilo terrenellus*; Ctum=*Chilo tumidicostalis*; Ed=*Emmalocera depressella*; Sc=*Sesamia calamistis*; Scrt=*Sesamia cretica*; Sg=*Sesamia grisescens*; Si=*Sesamia inferens*; Su=*Sesamia uniformis*; Se=*Scirpophaga excerptalis*; Sn=*Scirpophaga nivella*.

E = egg, L = larva, PP = pre pupa and P = Pupa. A question mark indicates an unknown or a doubtful status of record.

(1) Abdul Mannan & Iwahashi 1999; (2) Alam *et al.* 1972; (3) Alba 1989; (4) Alba 1990; (5) Alba 1991; (6) Anonymous 1954; (7) Appert 1973; (8) Appert *et al.* 1969; (9) Arakaki & Ganaha 1986; (10) Ashraf & Fatima 1996; (11) Barrion *et al.* 1987; (12) Betbeder-Matibet 1989; (13) Betbeder-Matibet & Malinge 1968; (14) Bhatt *et al.* 1996; (15) Bin & Johnson 1982; (16) Borah & Arya 1995; (17) Borah & Sarma 1995; (18) Box 1953; (19) Brenière *et al.* 1985; (20) Butani 1957; (21) Butani 1958; (22) Butani 1972; (23) Caresche 1962; (24) Carl 1962; (25) Chacko & Rao 1966; (26) Chandra & Avasthy 1988 (27) Cheng 1986; (28) Cheng & Chen 1998; (29) Cheng *et al.* 1987a; (30) Cheng *et al.* 1987b; (31) Cheng *et al.* 1995; (32) Cheng *et al.* 1997; (33) Cheng *et al.* 1999a; (34) Cheng *et al.* 1999b; (35) Cheng *et al.* 1999c; (36) CIBC 1966; (37) Chundurwar 1989; (38) Dai *et al.* 1988; (39) David *et al.* 1989; (40) David & Easwaramoorthy 1990; (41) Devi & Raj 1996; (42) Dey 1998; (43) Easwaramoorthy & Jayaraj 1987; (44) Easwaramoorthy & Nandagopal 1986; (45) Easwaramoorthy *et al.* 1983; (46) Easwaramoorthy *et al.* 1987; (47) Easwaramoorthy *et al.* 1992; (48) Easwaramoorthy *et al.* 1996a; (49) Easwaramoorthy *et al.* 1996b; (50) Easwaramoorthy *et al.* 1998; (51) Easwaramoorthy *et al.* 1999; (52) Facknath 1989; (53) Ganeshan 2000; (54) Ganeshan & Rajabalee 1997; (55) Godse & Nayak 1983; (56) Goebel *et al.* 2000; (57) Goel *et al.* 1983; (58) Greathead 1971; (59) Guo 1988; (60) Gupta *et al.* 1994; (61) Hashmi & Rahim 1985; (62) Imamura & Machimura 1976; (63) Imamura & Yamazaki 1975; (64) Jacquemard *et al.* 1985; (65) Jaipal & Chaudhary 1994; (66) Javier & Gonzalez 2000; (67) Jotwani 1982; (68) Jotwani & Verma 1969; (69) Kalshoven 1981; (70) Kajita & Drake 1969; (71) Kishore 1986; (72) Kumar & Kalra 1965; (73) Kuniata 1994; (74) Kuniata 2000; (75) Kuniata & Sweet 1994; (76) Kurian 1952; (77) Li 1970; (78) Li 1981; (79) Li 1985a; (80) Li 1985b; (81) Li 1990; (82) Liu *et al.* 1985; (83) Liu *et al.* 1987; (84) Liu *et al.* 1996; (85) Lloyd & Kuniata 2000; (86) Meenakanit *et al.* 1988; (87) Mehta & David 1980; (88) Mia & Iwahashi 1999; (89) Mohyuddin 1986; (90) Mohyuddin 1991; (91) Mohyuddin 1992; (92) Mohyuddin *et al.* 1972; (93) Mohyuddin 1990; (94) Moutia & Courtois 1952; (95) Mukunthan 1989; (96) Muzaffar & Inayatullah 1986; (97) Nair 1988; (98) Nagarkatti & Nair 1973; (99) Neupane *et al.* 1985; (100) Nickel 1964; (101) Nigam 1984; (102) Pan & Lim 1979; (103) Pandey et al 1997; (104)Pandya 1997; (105)Pati & Mathur 1986; (106)Pawar 1987; (107)Poinar et al 1992; (108)Rai et al 1999; (109)Rajabalee & Governdasamy 1988; (110) Rajendran 1999; (111) Rajendran & Gopalan 1995; (112) Rajendran & Hanifa 1996; (113) Rajendran & Hanifa 1997; (114) Rajendran & Hanifa 1998; (115) Rothschild 1970; (116) Samoedi 1989; (117) Samoedi & Wirioatmodjo 1986; (118) Sardana 1994; (119) Sardana 1997; (120) Sardana 2000; (121) Saxena 1992; (122) Selvaraj *et al.* 1994; (123) Sharma *et al.* 1966; (124) Shenhmar & Brar 1996; (125) Shenhmar & Varma 1988; (126) Shenhmar *et al.* 1990; (127) Shojai *et al.* 1995; (128) Singh *et al.* 1975; (129) Singhal *et al.* 2001; (130) Sivasankaran *et al.* 1990; (131) So & Okada 1989; (132) Sonan 1929; (133) Srikanth *et al.* 1999; (134) Suasa-ard 2000; (135) Suasa-ard & Charernsom 1995; (136) Subba Rao *et al.* 1969; (137) Sukhani 1986; (138) Sunaryo & Suryanto 1986; (139) Takano 1934; (140) Tan & Koh 1980; (141) Tanwar 1990; (142) Tanwar & Varma 1997; (143) Tuhan & Pawar 1983; (144) Ubandi & Sunaryo 1986; (145) Ubandi *et al.* 1988; (146) Varma & Saxena 1989; (147) Vinson 1942; (148) Wang *et al.* 1985; (149) Wen & Sun 1988; (150) Wen & Sun 1989; (151) Williams 1978; (152) Williams 1983; (153) Young 1982; (154) Zhang 1986.

References

Abdul Mannan M., Iwahashi O. 1999. Seasonal changes in infestation level of sugarcane by the pink borer, *Sesamia inferens* (Lepidoptera: Noctuidae), in relation to a parasitoid, *Cotesia flavipes* (Hymenoptera: Braconidae), on Okinawa Island. *Applied Entomology and Zoology* **34:** 429-434.

Alam M.M., Beg M.N., Ghani M.A. 1972. Introduction of *Apanteles* spp. against graminaceous borers into Pakistan. *Technical Bulletin, Commonwealth Institute of Biological Control* **15:** 1-10.

Alba M.C. 1989. Prospects and problems of biological control in sugarcane. *PHILSUTECH Proceedings* **36:** 144-153.

Alba M.C. 1990. *Use of natural enemies for controlling sugarcane pests in the Philippines.* FFTC-NARC International Seminar on the use of parasitoids and predators to control agricultural pests, Japan, 1989. National Agricultural Research Centre (NARC), 24pp.

Alba M.C. 1991. Utilization of *Trichogramma* for biological control of sugarcane borers in the Philippines. *Colloques de l'INRA* **56:**161-163.

Allsopp P.G., Samson P., Chandler K. 2000. Pest management, p. 91-108 *in:* **Hogarth D. M., Allsopp P. G. (eds)**, *Manual of canegrowing*, Brisbane, 436 p.

Anonymous. 1954. Review of agricultural entomology during the period 1948-1954, p. 281-284 *in: Report of the VIth commonwealth entomological conference*, Commonwealth Institute of Entomology, London, UK.

Appert J., Betbeder-Matibet M., Ranaivosoa H. 1969. Vingt années de lutte biologique à Madagascar. *Agronomie Tropicale* **26:** 555-572.

Appert J. 1973. Entomofaune parasitaire des foreurs des graminés à Madagascar. *Entomophaga* **18:** 77-94.

Arakaki N., Ganaha Y. 1986. Emergence pattern and mating behavior of *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae). *Applied Entomology and Zoology* **21:** 382-388.

Ashraf M., Fatima B. 1996. Success of *Trichogramma chilonis* (Ishii) for area-wide control of sugarcane borers in Pakistan. *Proceedings of Brighton Crop Protection Conference: Pests & Diseases* **1:** 385-388.

Austin A.D., Dangerfield P.C. 1992. Synopsis of Australian microgastrinae (Hymenoptera: Braconidae), with a key to genera and description of new taxa. *Invertebrate Taxonomy* **6:** 1-76.

Barrion A.T., Libetario E.M., Litsinger J.A. 1987. An earwig predator of Asian pink stem borer (PSB) on upland rice. *International Rice Research Newsletter* **12:** 21.

Bell A.F. 1934. *Annual Report.* Bureau of Sugarcane Experiment Stations, Qld. **34:** 71.

Betbeder-Matibet M., Malinge P. 1968. Un succès de la lutte biologique : contrôle de *Proceras sacchariphagus* Boj., Borer ponctué de la canne à sucre à Madagascar par un parasite introduit: *Apanteles flavipes* Cam. *Agronomie Tropicale* **22:** 1196-1220.

Betbeder-Matibet M. 1989. Biological control of sorghum stem borers, p. 89-93 in: *International Workshop on Sorghum Stem Borers*, 17-20 Nov 1987. ICRISAT, India.

Bhatt T.A, Vyas S.T., Mehta V.R., Patel K.K., Patel J.M. 1996. Natural parasitism in sugarcane root borer. *Bharatiya Sugar* 22: 37-39.

Bin F., Johnson N.F. 1982. Some new species of *Telenomus* (Hym., Scelionidae) egg-parasitoids of tropical pyralid pests (Lep., Pyralidae). *Redia* 65: 229-252.

Borah B.K., Arya M.P.S. 1995. Natural parasitization of sugarcane plassey borer (*Chilo tumidicostalis* HMPSN.) by braconid larval parasitoid in Assam. *Annals of Agricultural Research* 16: 362-363.

Borah B.K., Sarma K.K. 1995. Seasonal incidence of plassey borer, *Chilo tumidicostalis* Hmpsn. in ratoon sugarcane. *Plant Health* 1: 29-33.

Box H.E. 1953. *List of sugar-cane insects*. Commonwealth Institute of Entomology, London, 103 p.

Brenière J., Bordat D., Vercambre B., Hamza H., Renand M. 1985. Les opérations de lutte biologique contre le foreur du maïs *Chilo partellus* (Swinhoe) Lepidoptera, dans l'île de Ngazidja. *Agronomie Tropicale* 40: 157-166.

Butani D.K. 1957. A tachinid fly parasite of *Chilo zonellus* Swin. *Indian Journal of Entomology* 19: 62-63.

Butani D.K. 1958. Parasites and predators recorded on sugarcane pests in India. *Indian Journal of Entomology* 2: 270-282.

Butani D.K. 1972. Parasites and predators recorded on insect pests of sugarcane in India. *Indian Sugar* 22: 17-32.

Caresche L. 1962. Les insectes nuisibles de la canne à sucre dans l'Ile de la Réunion. *Agronomie Tropicale* 17: 632-646.

Carl K.P. 1962. Gramineous moth borers in West Pakistan. *Technical Bulletin CIBC* 2: 29-76.

Carnegie A.J.M., Conlong D.E., Graham D.Y. 1985. Recent introductions of parasitoids against *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Proceedings of the South African Sugar Technologists Association* 59: 160-163.

Chacko M.J., Rao V.P. 1966. *Centeterus alternecoloratus* Cushman? var., a pupal parasite of the graminaceous borer *Chilo partellus* (Swinhoe) and *Chilotraea auricilia* (Dudgeon). *Entomophaga* 11: 297-303.

Chandra J., Avasthy P.N. 1988. Effect of temperature variations on survival and development of *Sturmiopsis inferens* Tns. during winter months. *Indian Journal of Agricultural Research* 22: 159-163.

Cheng W.Y. 1986. Research on *Trichogramma chilonis* Ishii and its utilization for the control of sugarcane borers in Taiwan. *Plant Protection Bulletin, Taiwan* 28: 41-58.

Cheng W.Y., Chang C.H., Wang Z.T. 1987a. Occurrence of *Cotesia flavipes* Cameron in autumn sugarcane fields. *Report of the Taiwan Sugar Research Institute* 117: 31-41.

Cheng W.Y., Hung T.H., Hung J.K., Wang Z.T. 1987b. The occurrence of eggs of some sugarcane insect pests and egg parasitism by *Trichogramma chilonis* in the autumn-planted cane fields. *Report of the Taiwan Sugar Research Institute* 117: 13-30.

Cheng W.Y., Chen S.M., Wang Z.T. 1995. Differences in occurrence of *Trichogramma chilonis* and *T. ostriniae* between spring cane and sweet corn fields. *Report of the Taiwan Sugar Research Institute* 150: 23-41.

Cheng W.Y., Wang Z.T., Hung T.H. 1997. Seasonal occurrence of the eggs of borers and other *Trichogramma* hosts in the spring-planted cane fields. *Report of the Taiwan Sugar Research Institute* 156: 15-36.

Cheng W.Y., Chen S.M. 1998. Seasonal occurrence of *Telenomus beneficiens* Zehntner in spring cane of Taiwan. *Taiwan Sugar* 45: 20-26.

Cheng W.Y., Chen S.M., Wang Z.T. 1999a. Occurrence of *Meloboris sinicus* (Holmgren) in spring cane. *Report of the Taiwan Sugar Research Institute* 165: 15-28.

Cheng W.Y., Chen S.M., Wang Z.T. 1999b. Survey of larval and pupal parasitoids of cane borers in spring cane. *Report of the Taiwan Sugar Research Institute* 164: 1-14.

Cheng W.Y., Chen S.M., Singh V., Kumar V. 1999c. Occurrence of *Telenomus beneficiens* var. *elongatus*, an egg parasitoid of the sugarcane top borer in spring cane. *Proceedings of the XXIII ISSCT Congress, India* 2: 553-558.

Chundurwar R.D. 1989. Sorghum stem borers in India and Southeast Asia, p. 19-25 in: *International Workshop on Sorghum stemborers*, ICRISAT, India.

CIBC. 1966. *Final report on Investigations on the natural enemies of corn borer*. Pakistan Station Commonwealth Institute of Biological Control, Rawalpindi.

Conlong D.E. 1997. Biological control of *Eldana saccharina* Walker in South African sugarcane: Constraints identified from 15 years of research. *Insect Science and its Application* 17: 69-78.

Conlong D.E., Goebel R. 2002. Biological control of *Chilo sacchariphagus* (Lepidoptera: Crambidae) in Mozambique: The first steps. *Proceedings of the South African Sugarcane Technologists Conference* 76: 310 - 320.

Dai K., Zhang L., Ma Z., Zhong L., Zhang Q., Cao A., Xu K., Li Q., Gao Y. 1988. Research and utilization of artificial host egg for propagation of parasitoid *Trichogramma* and other egg parasites. *Colloques de l'INRA* 43: 311-318.

David H., Easwaramoorthy S. 1990. Biological control of *Chilo* spp. in sugar-cane. *Insect Science and its Application* 11: 733-748.

David H., Easwaramoorthy S., Kurup N.K., Shanmugasundaram M., Santhalakshmi G. 1989. A simplified mass culturing technique for *Sturmiopsis inferens* Tns. *Journal of Biological Control* 3: 1-3.

Devi N., Raj D. 1996. Extent of parasitization of *Chilo partellus* (Swinhoe) on maize by *Apanteles* sp. in mid hill zone of Himachal Pradesh (India). *Journal of Entomological Research* 20: 171-172.

Dey D. 1998. New records of four braconid (Hymenoptera: Braconidae) genera from India. *Shashpa* 5: 103-104.

Easwaramoorthy S., Nandagopal V., David H., Goel S.C. 1983. Seasonal importance of parasites and predators on sugarcane internode borer, *Chilo sacchariphagus* indicus (K.), p 55-61 in: Goel S.C (ed.) *Insect ecology and resource management*, Sanatan Dharm College, Muzaffarnagar.

Easwaramoorthy S., Nandagopal V. 1986. Life tables of internode borer, *Chilo sacchariphagus indicus* (K.), on resistant and susceptible varieties of sugarcane. *Tropical Pest Management* 32: 221-228.

Easwaramoorthy S., Jayaraj S. 1987. Cross infectivity of granulosis viruses infecting *Chilo infuscatellus* Snell and *C. sacchariphagus indicus* (Kapur). *Journal of Entomological Research* 11: 170-174.

Easwaramoorthy S., Kurup N., David H. 1987. Occurrence of *Nosema* sp. in sorghum stem borer, *Chilo partellus* Swinhoe. *Madras Agricultural Journal* 74: 332.

Easwaramoorthy S., David H., Shanmugasundaram M., Santhalakshmi G., Kumar R. 1992. Laboratory and field evaluation of *Allorhogas pyralophagus* for the control of lepidopterous borers infesting sugarcane in tropical India. *Entomophaga* 37: 613-619.

Easwaramoorthy S., Srikanth J., Santhalakshmi G. 1996. Seasonal occurrence of the fungus *Hirsutella nodulosa* Petch and granulosis virus of sugarcane internode borer *Chilo sacchariphagus indicus* (Kapur). *Entomon* 21: 205-209.

Easwaramoorthy S., Srikanth J., Santhalakshmi G., Kurup N.K. 1996. Life history and prey acceptance of commonly occurring spiders in sugarcane ecosystem. *Journal of Biological Control* 10: 39-47.

Easwaramoorthy S., Strongman D.B., Santhalakshmi G. 1998. Record of *Hirsutella nodulosa* Petch from *Chilo sacchariphagus* indicus (Kapur), sugarcane internode borer in India. *Journal of Biological Control* 11: 79-80.

Easwaramoorthy S., Kurup N.K ., Santhalakshmi G. 1999. Record of *Bacillus cereus* infection on *Sturmiopsis inferens* Tns. puparia. *Insect Environment* 5: 71-72.

Facknath S. 1989. Biological control of sugar-cane pests in Mauritius: A case study. *Insect Science and its Application* 10: 809-813.

Ganeshan S., Rajabalee A. 1997. Parasitoids of the sugarcane spotted

borer, *Chilo sacchariphagus* (Lepidoptera: Pyralidae), in Mauritius. *Proceedings of the Annual Congress South African Sugar Technologists Association* **71**: 87-90.

Ganeshan S. **2000**. Biological control of sugarcane pests in Mauritius: current status and future prospects, p. 3-9 *in: Proceedings of the IV Sugarcane Entomology Workshop*, ISSCT.

Godse D.B., Nayak P. **1983**. Nuclear polyhedrosis of *Sesamia inferens* (Noctuidae: Lepidoptera) the pink stemborer of rice. *Current Science* **52**: 682-683.

Goebel R., Fernandez E., Beugue J.M., Tibere R., Alauzet C. **2000**. Predation and varietal resistance as important components of integrated protection of the sugarcane stemborer *Chilo sacchariphagus* (Bojer) (Lepidoptera: Pyralidae) in Reunion, p. 51-56 *in: Proceedings of the IV Sugarcane Entomology Workshop*, ISSCT, Thailand

Goel S.C., Roy T.C.D., Bains S.S. **1983**. Key mortality factors in the population dynamics of sugarcane top borer, *Tryporyza nivella* (Fabr) in the Punjab, p. 270-283 *in:* Goel S.C. (ed.) *Insect ecology and resource management*, Sanatan Dharm College, Muzaffarnagar.

Greathead D.J. **1971**. *A review of biological control in the Ethiopian region. Commonwealth Institute of Biological Control.* Technical Communication No. 5. Commonwealth Agricultural Bureau, Farnham Royal, UK, 162 pp.

Guo M.F. **1988**. New method of *Trichogramma* utilization. In *Trichogramma and other egg parasites. Colloques de l'INRA* **43**: 469-476.

Gupta S.C, Yazdani S.S, Hameed S.F., Agarwal M.L. **1994**. Seasonal prevalence of larval parasitoids of sugarcane top borer, *Scirpophaga excerptalis* in North Bihar (India). *Journal of Entomological Research* **18**: 83-87.

Hashmi A.A., Rahim A. **1985**. Pest management model for sugar-cane borers in Sind, Pakistan. *International Pest Control* **27**: 88-91.

Imamura K., Machimura N. **1976**. Studies on the parasite, *Apanteles chilonis* Munakata, on the rice stem borer, *Chilo suppressalis* Walker. *Proceedings of the Association for Plant Protection of Hokuriku* **24**: 45-50.

Imamura K., Yamazaki S. **1975**. Studies on the parasite, *Apanteles chilonis* Munakata, on rice stemborer, *Chilo suppressalis* Wlker: Emerging habits. *Proceedings of the Association for Plant Protection of Hokuriku* **23**: 68-72.

Jacquemard P., Croizier G., Amargier A., Veyrunes J.C., Croizier L., Bordat D., Vercambre B. **1985**. Presence of three viruses in *Sesamia calamistis* Hampson (Lepidoptera: Agrotidae) on the island of Reunion. *Agronomie Tropicale* **40**: 66-71.

Jaipal S., Chaudhary J.P. **1994**. Laboratory studies on temperature tolerance in *Sturmiopsis inferens* Tns. (Tachinidae: Diptera). *Journal of Insect Science* **7**: 93-94.

Javier P., Gonzalez P. **2000**. Sugarcane borers. *Philsurin update* **4**: 3.

Jarvis D. **1927**. Notes on insects damaging sugarcane in Queensland. *BESE Bulletin* **3**: 94.

Jones E.L. **1966**. Rice stem borer *Bathytrica truncata*. N.S.W. Department of Agriculture, Entomology branch. *Annual Report* **1965/66**: 14-15.

Jotwani M.G., Verma K.K. **1969**. *Menochilus sexmaculata* (Fabricius) as a predator of sorghum stemborer, *Chilo zonellus* (Swinh.). *Indian Journal of Entomology* **31**: 84-85.

Jotwani M.G. **1982**. Factors reducing sorghum yields: Insect pests, p. 251-255 *in: Sorghum in the eighties: proceedings of the International Symposium on Sorghum*, 15th – 21st July 1982, Vol. I, ICRISAT, Patancheru, India.

Kajita H., Drake E.F. **1969**. Biology of *Apanteles chilonis* and *A. flavipes* (Hymenoptera: Braconidae), parasites of *Chilo suppressalis*. *Mushi* **42**: 163-179.

Kalshoven, L. G. E. **1981**. *Pests of crops in Indonesia. (Revised and translated by P.A. van der Laan)* P.T. Ichtiar Baru-van Hoeve, Jakarta, 701 p.

Kfir R. **1997**. Natural control of the cereal stemborers *Busseola fusca* and *Chilo partellus* in South Africa. *Insect Science and its Application* **17**: 61-67.

Kishore P. **1986**. Studies on natural enemies of the spotted stem borer (*Chilo partellus* (Swinhoe)). *Sorghum Newsletter* **29**: 65-66.

Kumar S., Kalra A.N. **1965**. Attack of the pink borer, *Sesamia inferens* WLK. As cane borer in Rajasthan. *Indian Sugarcane* **9**: 154-155.

Kuniata L.S. **1994**. Pest status, biology and effective control measures of sugarcane stalk borers in the Australian, Indonesian and Pacific Island sugarcane growing regions, p 83-96 *in:* Caregie A. & Conlong D. (eds.), *Proceedings of the second ISSCT Sugarcane Entomology Workshop*, Mount Edgecombe, South Africa.

Kuniata L.S., Sweet C.P. **1994**. Management of *Sesamia grisescens* Walker (Lep.: Noctuidae), a sugar-cane borer in Papua New Guinea. *Crop Protection* **13**: 488-493.

Kuniata L.S. **2000**. Integrated management of sugarcane stemborers in Papua New Guinea, p. 37-50 *in: Proceedings of the IV Sugarcane Entomology Workshop, ISSCT.*

Kurian C. **1952**. Description of four new and record of one known Bethyloidea (Parasitic Hymenoptera) from India. *Agra University Journal of Research* **1**: 63-72.

Li C.S. **1970**. Some aspects of the conservation of natural enemies of rice stem borers and the feasibility of harmonizing chemical and biological control of these pests in Australia. *Mushi* **44**: 15-23.

Li J.K. **1981**. *Barathra brassicae* (L.) a new host of *Vulgichneumon leucaniae* Uchida. *Insect Knowledge (Kunchong Zhishi)* **18**: 253.

Li C.S. **1985a**. Sugarcane insect pests with special reference to moth borers in the Markham Valley, Papua New Guinea. *Mushi* **50**: 13-18.

Li W.J. **1985b**. Efficacy of *Chelonus munakatae* against the millet borer. *Chinese Journal of Biological Control* **1**: 41.

Li C.S. **1990**. Status and control of *Chilo* spp., their distribution, host range and economic importance in Oceania. *Insect Science and its application* **11**: 535-539.

Liu Z.C, Sun Y.R., Wang Z.Y., Liu J.F., Zhang L.W., Zhang Q.X., Dai K.J., Gao Y.G. **1985**. Field release of *Trichogramma confusum* reared on artificial host eggs against sugarcane borers. *Chinese Journal of Biological Control* **3**: 2-5.

Liu W.H., Guo M.F., Han S.C., Wu H.H., Li L.Y. **1987**. Inundative release of *Trichogramma nubilale* Ertle & Davis to control sugarcane borers in large areas. *Chinese Journal of Biological Control* **3**: 149-151.

Liu Z., Liu J., Wang C., Yang W., Liu Z., Liu J., Wang C., Yang W. **1996**. The role of biological control in integrated control of sugarcane insect pests, p. 226-230 *in Proceedings of the International Symposium on „The use of Biological Control Agents under Integrated Pest Management" Biological pest control in systems of integrated pest management*. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taipei, Taiwan.

Lloyd P., Kuniata L. **2000**. The control of *Sesamia* at Ramu. *Sugarcane International*, April **2000**: 10-17.

Meenakanit L., Sampeth T., Disthaporn S. **1988**. Field trials of *Trichogramma chilotraeae* used to control *Chilo infuscatellus*. *Trichogramma and other egg parasites: Colloques de l'INRA* **43**: 499-500.

Mehta O.K., David H. **1980**. A granulosis virus disease of sugar-cane internode borer. *Madras Agricultural Journal* **67**: 616-619.

Mia A., Iwahashi O. **1999**. Seasonal changes in infestation level of sugarcane by the pink borer, *Sesamia inferens* (Lepidoptera: Noctuidae), in relation to a parasitoid, *Cotesia flavipes* (Hymenoptera: Braconidae), on Okinawa Island. *Applied Entomology and Zoology* **34**: 429-434.

Mohyuddin A.I., Mushtaq M., Attique M.R. **1972**. *Research on bionomics, biology and control of maize stem borer and its enemies*. Annual Report Pakistan Station, CIPC Rawalpindi.

Mohyuddin A.I. **1986**. *Report on a visit to Indonesia to advise on management of sugarcane pests*. PARC-CIBC Station; Rawalpindi.

Mohyuddin AI. **1990**. Biological control of *Chilo* spp. in maize, sorghum and millet. *Insect Science and its Application* **11**: 721-732.

Mohyuddin A.I. 1991. Utilization of natural enemies for the control of insect pests of sugar-cane. *Insect Science and its Application* 12: 1-3, 19-26.

Mohyuddin A.I. 1992. Implementation of integrated pest management of sugarcane pests in Pakistan, p. 73-84 *in*: *Integrated pest management in the Asia Pacific region*, CAB International, Wallingford, UK.

Moutia LA., Courtois CM. 1952. Parasites of the moth-borers of sugarcane in Mauritius. *Bulletin of Entomological Research* 43: 325-359.

Mukunthan N. 1989. Life tables of sugarcane top borer, *Scirpophaga excerptalis* Wlk. *Insect Science and its Application* 10: 269-276.

Muzaffar N., Inayatullah C. 1986. Role of salivary glands of pyralid borer larvae in host selection by *Apanteles flavipes* (Cameron) (Hym.: Braconidae). *Journal of Agricultural Research, Pakistan* 24: 203-205.

Nagarkatti S., Nair K.R. 1973. The influence of wild and cultivated Graminae and Cyperacae on populations of sugarcane borers and their parasites in north India. *Entomophaga* 18: 419-430.

Nair K.R. 1988. Field parasitism by *Apanteles flavipes* Cam. (Hymenoptera: Braconidae) on *Chilo partellus* Swinh. in *Coix lachryma-jobi* L. and *Chilo auricilius* (Dudgn.) in sugarcane in India. *Entomon* 13: 283-287.

Neupane F.P., Coppel H.C., Chapman R.K. 1985. Bionomics of the maize borer *Chilo partellus* (Swinhoe) in Nepal. *Insect Science and its application* 6: 547-553.

Ngi Song A.J., Overholt W.A., Ayertey J.N. 1995. Suitability of African gramineous stemborer for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Environmental Entomology* 24: 978-984.

Nickel J.L. 1964. *Biological control of rice stem borers: A feasibility study*. Technical Bulletin No.2, IRRI, Laguna, 111 p.

Nigam H. 1984. Record of *Apanteles ruficrus* Hal. (Hymenoptera: Braconidae) as a new larval parasite of sugarcane stalk borer *Chilo auricilius* Dudg. *Indian Journal of Entomology* 46: 363.

Overholt W.A., Ngi_Song A.J., Omwega C.O., Kimani S.N., Mbapila J., Sallam M.N., Ofomata V. 1997. A review of the introduction and establishment of *Cotesia flavipes* Cameron in East Africa for biological control of cereal stemborers. *Insect Science and its Application* 17: 79-88.

Pan Y.C., Lim G.T. 1979. The biological control of sugarcane borers in Gula Perak plantation, Malaysia. *Malaysian Journal of Agriculture* 52: 129-134.

Pandey K.P., Singh R.P., Saxena A.K., Singh R.G. 1997. Occurrence of Gurdaspur borer, *Acigona steniellus* Hamp in eastern Uttar Pradesh. *Indian Sugar* 47: 137-138.

Pandya H. 1997. Biological control of sugarcane pests. *Cooperative Sugar* 28: 684-686.

Pati P., Mathur K.C. 1986. *Amyotea* (*Asopus*) *malabarica* (Fabricius), a predatory bug on leaf feeding pests of rice. *Oryza* 23: 200-201.

Pawar A.D. 1987. Biological control of sugarcane pests in India. *Plant Protection Bulletin* 39: 1-6.

Poinar G.O., Karunakar G.K., David H. 1992. *Heterorhabditis indicus* n. sp. (Rhabditida: Nematoda) from India: separation of Heterorhabditis spp. by infective juveniles. *Fundamental and Applied Nematology* 15: 467-472.

Polaszek A. 1998. *African cereal stem borers: economic importance, taxonomy, natural enemies and control*. CAB International, Wallingford, UK, 530 p.

Rai A.K., Khan M.A., Kaur S. 1999. Biological control of stalk borer, *Chilo auricilius* Dudg. *Sugarcane belt of U.P. Shashpa* 6: 59-62.

Rajabalee M., Governdasamy M. 1988. Host specificity and efficacy of *Apanteles flavipes* and *A. sesamiae* (Hymenoptera: Braconidae) parasites of sugar can moth borers in Mauritius. *Revue Agricole et Sucrière de l'Ile Maurice* 67: 78-80.

Rajendran B. 1999. Field parasitization of sugarcane internode borer by egg parasitoid *Telenomus beneficiens* Zehnt. *Entomon* 24: 285-287.

Rajendran B., Gopalan M. 1995. Efficacy of *Trichogramma chilonis* and *Telenomus beneficiens* on sugarcane internode borer. *Madras Agricultural Journal* 82: 320-321.

Rajendran B., Hanifa A. 1996. Efficacy of different release techniques of *Trichogramma chilonis* Ishii, parasitoid on sugarcane internode borer. *Indian Journal of Plant Protection* 24: 98-101.

Rajendran B., Hanifa A. 1997. Effect of insecticides on the emergence of egg parasitoid *Trichogramma chilonis* Ishii. in sugarcane field. *Cooperative Sugar* 29: 27-30.

Rajendran B., Hanifa A.M. 1998. Efficacy of different techniques for the release of *Trichogramma Chilonis* Ishii, parasitising sugarcane internode borer, *Chilo sacchariphagus indicus* (Kapur). *Journal of Entomological Research* 22: 355-359.

Rodriguez-del-Bosque L.A., Browning H.W., Smith J.W. 1990. Seasonal parasitism of corn stalkborers (Lepidoptera: Pyralidae) by indigenous and introduced parasites in Northern Mexico. *Environmental Entomology* 19: 393-402.

Rothschild G.H.L. 1970. Parasites of rice stemborers in Sarwak (Malaysian Borneo). *Entomophaga* 15: 21-51.

Samoedi D. 1989. The impacts of mass liberation of *Diatraeophaga striatalis* Towns. on population and intensity of infestation of sugarcane moth-borer, *Chilo auricilius* Dudg. in central Java. *Gula Indonesia* 15: 46-48.

Samoedi D., Wirioatmodjo B. 1986. Population dynamics of the sugarcane top borer, *Tryporyza nivella intacta* Sn., and its parasitoids in Central Java, Indonesia. *ISSCT* 19: 596-603.

Sardana H.R. 1994. New record of parasite of sugarcane root borer, *Emmalocera depressella* Swinhoe. *Bulletin of Entomology New Delhi* 35: 150-151.

Sardana H.R. 1997. Occurrence of entomophagous fungi on sugarcane root borer, *Emmalocera depressella* Swinhoe. *Annals of Agricultural Research* 18: 104.

Sardana H.R. 2000. Integrated management of sugarcane root borer, *Emmalocera depressella* Swinhoe. *Cooperative Sugar* 32: 271-274.

Saxena H. 1992. *Spathius elaboratus* Wilkinson (Hymenoptera: Braconidae: Doryctinae) - a new larval parasite of gurdaspur borer, *Acigona steniella* (Hampson) from Uttar Pradesh. *Indian Journal of Entomology* 54: 482.

Selvaraj A., Sundara-Babu P.C., Babu P.C. 1994. Release of different doses of *Trichogramma* and its effect on internode borer, yield and quality of sugarcane. *Sugarcane* 2: 22-23.

Sharma A.K., Saxena J.D., Subba Rao B.R. 1966. A catalogue of the hymenopterous and dipterous parasites of *Chilo zonellus* (Swinhoe) (Crambidae: Lepidoptera). *Indian Journal of Entomology* 28: 510-542.

Shenhmar M., Brar K.S. 1996. Efficacy of two strains of *Cotesia flavipes* (Cameron) for the control of sugarcane borers. *Indian Sugar* 45: 877-879.

Shenhmar M., Varma G.C. 1988. Some observations on biology and rearing technique of *Rhaconotus signipennis* (Walker) (Braconidae: Hymenoptera). *Journal of Research, Punjab Agricultural University* 25: 67-69.

Shenhmar M., Verma G.C., Brar K.S. 1990. Studies on the establishment of *Allorhogas pyralophagus* Marsh. (Braconidae: Hymenoptera) on sugarcane borers in the Punjab. *Journal of Insect Science* 3: 53-56.

Shojai M., Abbas P.H., Nasrollahi A., Labbafi Y. 1995. Technology and biocenotic aspects of integrated biocontrol of corn stem borer: *Sesamia cretica* Led. (Lep. Noctuidae). *Journal of Agricultural Sciences Islamic Azad University* 1: 5-32.

Singh B., Dhaliwal J.S., Battu G.S., Atwal A.S. 1975. Population studies on the maize-borer *Chilo partellus* (Swinhoe) in the Punjab. III. Role of parasitization by *Apanteles flavipes* (Cameron) in the population build-up. *Indian Journal of Ecology* 2: 115-124.

Singhal R.C., Gupta M.R., Narayan D. 2001. Eco-friendly approach for minimising populations of sugarcane stalk borer (*Chilo auricilius*) in the Tarai Belt of Uttar Pradesh, India. *ISSCT* 24: 374-377.

Sivasankaran P., Easwaramoorthy S., David H. 1990. Pathogenicity and host range of *Beauveria* nr. *bassiana*, a fungal pathogen of *Chilo infuscatellus* Snellen. *Journal of Biological Control* 4: 48-51.

Smith J.W. Jr., Wiedenmann, R.N., Overholt W.A. 1993. *Parasites of lepidopteran stemborers of tropical gramineous plants*. ICIPE Science Press, Nairobi, 89 p.

Smith J.W. Jr. 1994. Publications associated with stalk borers and Texas, p. 75-82 *in:* **Caregie A., Conlong D. (eds.),** *Proceedings of the second ISSCT Sugarcane Entomology Workshop,* Mount Edgecombe, South Africa.

Smith J.W. Jr., Wiedenmann R.N. 1997. Foraging strategies of stemborer parasites and their application to biological control. *Insect science and its Application* 17: 37-49.

So D.P., Okada M. 1989. Cross infectivity of nuclear polyhedrosis viruses to the common armyworm, *Pseudaletia separata. Korean Journal of Applied Entomology* 28: 10-15.

Sonan J. 1929. A few Host-Known Ichneumonidae found in Formosa. *Transactions of the Natural History Society of Formosa* 19: 415-425.

Srikanth J., Easwaramoorthy S., Shanmugasundaram M., Kumar R. 1999. Seasonal fluctuations of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) parasitism in borers of sorghum and sugarcane in southern India. *Insect Science and its Application* 19: 65-74.

Suasa-ard W. 2000. *Chilo tumidicostalis* (Hampson) (Lepidoptera: Pyralidae) and its natural enemies in Thailand, p. 10-16 *in: Proceedings of the IV Sugarcane Entomology Workshop, ISSCT, Thailand.*

Suasa-ard W., Charernsom K. 1995. Natural enemy complex of sugarcane moth borers in Thailand. *ISSCT* 21: CCCLIV, Poster paper.

Subba Rao B., Singh R., Saxena J., Sharma A.K. 1969. Bionomics of *Apanteles flavipes* (Cameron) a parasite of *Chilo zonellus* (Swinhoe) at Delhi with special reference to the mode of overwintering of the parasite. *Indian Journal of Entomology* 31: 7-12.

Sukhani T.R. 1986. Insect pest management in sorghum. *Plant Protection Bulletin* 38: 57-62.

Sunaryo, Suryanto S.J. 1986. Augmentation of local and introduction of Thailand strain of *Cotesia flavipes* (Cam.) in Gunung Madu Plantations, Indonesia, p. 153-159 *in: Proceedings of the Annual Convention, Pakistan Society of Sugar Technologists (22nd).*

Takano S. 1934. Hymenopterous parasites of lepidopterous pests of sugarcane in Formosa and their bibliography. *Journal of Formosa Sugar Plant Association* 11: 454-466.

Tan S.W., Koh H.L. 1980. Integrated control of sugarcane borers in the Northwest Peninsula Malaysia and Lampung, Sumatra, Indonesia. *ISSCT* 17: 1693-1703.

Tanwar R.K. 1990. Biology of *Elasmus zehntneri* Ferriere, an ectolarval parasitoid of the sugarcane top borer, *Scirpophaga excerptalis* Walker. *Journal of Biological Control* 4: 120-121.

Tanwar R.K., Varma A. 1997. *Scirpophaga excerptalis* Walker infestation in relation to its natural parasitoids and sugarcane cultivars in eastern Uttar Pradesh. *Uttar Pradesh Journal of Zoology* 17: 33-37.

Tuhan N.C., Pawar A.D. 1983. Life history, host suitability and effectiveness of *Trichogramma chilonis* (Ishii) for controlling sugarcane borers in the Punjab. *Journal of Advanced Zoology* 4: 71-76.

Ubandi H., Sunaryo. 1986. Introduction of *Allorhogas pyralophagus* Marsh (Braconidae) in Lampung (Indonesia) with preliminary notes on its biology. *Proceedings of the International Society of Sugarcane Technologists* 19: 563-567.

Ubandi H., Sunaryo, Suryanto S., Suroyo, Mohyuddin A. 1988. Notes on biology of *Elasmus zehntneri* Ferriere and its augmentation for controlling top borer *Scirpophaga nivella* (F.) at Gunung Madu Plantations, Indonesia, *Pakistan Society of Sugar Technologists* 23: 243-254.

van Hamburg H., Hassell M. 1984. Density dependence and the augmentative release of egg parasitoids against graminaceous stalk borers. *Ecological Entomology* 9: 101-108.

Varma A., Saxena H. 1989. Field recovery of an exotic parasite, *Allorhogas pyralophagus* Marsh against sorghum borer *Chilo partellus* Swinhoe. *Indian Journal of Plant Protection* 17: 101-102.

Vinson J. 1942. Biological control of *Diatraea mauriciella* in Mauritius. I. Investigation in Cylon in 1939. *Bulletin of Entomological Research* 33: 45.

Wang G.R., Liu Z.C., Wang Z.Y., Sun S.R., Jiang Q.R., Liang Y.F., Zhen Q., Zheng J.C. 1985. Studies on the dominant species of *Trichogramma* in sugarcane fields in Guangdong Province and the effect of releasing exotic species. *Natural Enemies of Insects, Kunchong Tiandi* 7: 13-18.

Way M.J., Turner P.E.T. 1999. The spotted sugarcane borer, *Chilo sacchariphagus* (Lepidoptera: Pyralidae: Crambinae), in Mozambique. *Proceedings of the South African Sugarcane Technologists Association* 73: 112-113.

Wen C.S., Sun C.X. 1989. Notes on Microsporidia (Protozoa Microspora) from the silkworm *Bombyx mori* L. and the sugarcane stem borer *Chilo infuscatellus* Snella in China. *Scientia Agricultura Sinica* 22: 15-19.

Wen J.Z., Sun C.X. 1988. Two new species of *Nosema* (Microspora: Nosematidae). *Acta Zootaxonomica Sinica* 13: 105-111.

Williams J.R. 1978. *An annotated check list of the invertebrates (insects, mites, nematodes) of sugarcane in Mauritius.* Occasional paper No. 31. Mauritius Sugar Industry Research institute, 22 p.

Williams J.R. 1983. The sugarcane stem borer (*Chilo sacchariphagus*) in Mauritius. *Revue Agricole et Sucriere de l'Ile Maurice* 62: 5-23.

Young G.R. 1982. Recent work on biological control in Papua New Guinea and some suggestions for the future. *Tropical Pest Management* 28: 107-114.

Zhang Z.Q. 1986. A study on biological and ecological characteristics of *Apanteles ruficrus* Haliday. *Natural Enemies of Insects* 8: 84-89.

History of the systematics of the *Sesamia sensu lato* group of African noctuid stem borers of monocotyledonous plants (Lepidoptera)

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Abstract. From the description of the genus *Sesamia* Guenée in 1852 to the latest work on the African species, the history of the systematics of this difficult group of African noctuid stem borers is recounted. The misidentifications that confused the taxonomy of these taxa and the new light shed when genitalia observation was first used are described. Some difficulties that still remain in classifying the 157 species described to date are emphasised and possible improvement by the combined use of morphological and molecular analyses is stressed.

Résumé. Histoire de la systématique des noctuelles africaines (groupe *Sesamia sensu lato*) foreuses de plantes monocotylédones (Lepidoptera : Noctuidae). L'histoire de la systématique du difficile groupe des noctuelles africaines foreuses de monocotylédones est présentée, depuis la description du genre *Sesamia* Guenée en 1852 jusqu'aux dernières diagnoses d'espèces. Les erreurs d'identification qui ont contribué à créer la confusion dans ce groupe sont décrites ainsi que les éclaircissements apportés par l'introduction de l'étude des genitalia. Les quelques difficultés qui demeurent pour classer les 157 espèces actuellement décrites sont indiquées. L'utilisation conjointe de l'observation morphologique et de l'outil moléculaire est préconisée afin d'améliorer la connaissance systématique du groupe.

Keywords: Lepidoptera, Noctuidae, stem borer, systematics, Africa.

Noctuid stem borers of monocotyledonous plants can be divided into two groups, which differ not only in their morphology but also in their geographical distribution and their evolutionary history. One of these groups is present mainly in temperate regions: it is comprised of 20 genera such as *Nonagria* Ochsenheimer 1816, *Archanara* Walker 1866, *Arenostola* Hampson 1910, *Apamea* Ochsenheimer 1816, *Oligia* Hübner 1816, and others. Some of these are found in Africa. For instance, *Apamea* includes 131 species among which eight are from Africa (two from Eritrea, three from Madagascar, and one each from Kenya, Mauritius and Reunion); *Oligia* is highly diversified in Africa with 34 species out of a total of 71 species in the genus described from various countries of Africa (the others are mainly from North America); *Mesoligia* Boursin 1965, a small genus close to *Oligia*, includes five species, two of which are described from Tanzania, two from Europe and one from Saudi Arabia. These genera are either mainly holoarctic or cosmopolitan and likely have a paleoarctic origin. The situation is quite different for the intertropical group of stem borers, the so-called *Sesamia sensu lato* group (Holloway 1998). It has typical particular morphological characteristics, and is limited to the intertropical regions of the Old World with the exception of some rare species of *Sesamia* Guenée 1852, that can be found in Mediterranean regions. The purpose of the present paper is to recount the history of the systematics of this intertropical group and to examine how knowledge progressed and was sometimes stopped for decades. The difficulties in deciphering the taxonomic relationships that still remain today are highlighted.

Results

The origins

Although two species of African noctuid stem borers were described in 1790 (*Phalaena vuteria* Stoll, later named *Speia vuteria*) and in 1827 (*Cossus nonagrioides* Lefebvre, the future *Sesamia nonagrioides*), the systematics of intertropical noctuid stem borers was really born in 1852 with the creation by Guenée (1852) of the genus *Sesamia* , with the species type *S. nonagrioides*. From this time, this group has been distinguished and recognised as a special taxonomic entity. This was the only contribution of Guenée to this group of intertropical noctuids, and it concerned the only species present in southern Europe. A few other descriptions of African noctuid stem borer species appeared at the end of the century, and preceded the monumental work of Hampson (1910) done at the British Museum during the last decade of the 19th Century and the first 20 years of the 20th Century.

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Hampson comes into the picture

Hampson (1910), who was the great specialist of moths at the turn of the 20th Century, described four new genera of African stem borers: *Acrapex* Hampson 1894 (fig. 1), *Conicofrontia* Hampson 1902 (fig. 2), *Phragmatiphila* Hampson 1908 and *Calamistis* Hampson 1908. The only African *Phragmatiphila* species was later transferred to *Poeonoma* Tams & Bowden 1953 (Tams & Bowden 1953), while the genus *Calamistis* is no longer used. The species type of this latter genus was *C fusca*
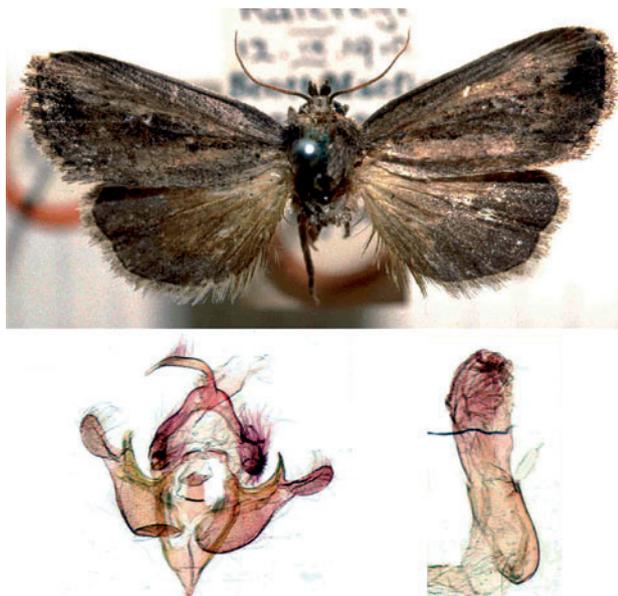

Figure 1

Acrapex hemiphlebia (Hampson 1914) (A: adult.; B: male genitalia; C: aedeagus).

Figure 2

Conicofrontia diamesa (Hampson 1920) (A: adult; B: male genitalia; C aedeagus).

Hampson 1902, a species that Hampson described in 1902 as *Sesamia fusca*. In 1904, however, Thurau described what he thought to be a new species, for which he created the genus *Busseola* Thurau 1904: *B. sorghicida*. This species was a synonym of *S. fusca*. Therefore, since in systematics there is a priority rule when both species are recognized as synonyms, the species took the name *fusca* and kept the genus name *Busseola*. Since *Calamistis* had the species type *C. fusca*, all the species in this genus were transferred to *Busseola*, which had priority. In his catalogue of the Lepidoptera Phalaenae in the British Museum, Hampson (1910) described these new genera and transferred some species to them. This resulted altogether in one species in *Phragmatiphila*, four species in *Conicofrontia*, eight species in *Acrapex*, seven in *Calamistis* and 10 in *Sesamia*. By 1920 Hampson had added three new African species to *Sesamia*, four to *Busseola* and six to *Acrapex* (or that were later transferred to *Acrapex*). Hampson alone described 40 new species of African noctuid stem borers, which is the major contribution to this group. The only other important work in the field before the Second World War was that of Janse (1939), who described six species of *Acrapex* and two species of *Sesamia* from South Africa. The total number of species described was by then 63, but later two of them sank as synonyms, so the number of valid species at the time amounted to 61. Fourteen years passed before a substantial new event occurred.

Tams & Bowden, and the revision of African Sesamia

The work by Tams & Bowden (1953) and the subsequent one by Bowden (1956) mark a turning point in the African studies of noctuid stem borers. These authors shed new light on the topic thanks to the observation of the genitalia, which enabled an easier separation of the species. The first use of genitalia was by Janse (1939), but in some cases he did not observe the types and made some mistakes. For instance, he attributed the name *Sesamia calamistis* Hampson 1910 to a different species that was later named *Sesamia jansei* by Tams & Bowden in 1953; he also presented pictures of genitalia often in a profile view that did not enable easy species identification. Tams & Bowden (1953) checked the genitalia of many types in the British Museum, and presented them in a fashion that enabled, in most cases, a fairly easy determination of the species by field entomologists. This accurate study enabled them to demonstrate a major error in Hampson's work. In the Catalogue, this last author has put in synonymy *Sesamia nonagrioides* and *Phalaena vuteria* Stoll, which has priority, resulting in *S. vuteria* becoming the new species type of the genus *Sesamia* (Hampson 1910). Tams & Bowden (1953) showed that *Phalaena vuteria* was a different species and did not belong at all to *Sesamia* and thus created the genus *Speia* Tams & Bowden 1953 (fig. 3) for it. *S. nonagrioides* was then rehabilitated, as well as *Sesamia madagascariensis* Saalmüller 1891, which also had been sunk as a synonym of *S. vuteria* by Hampson (1910). Tams and Bowden created two other genera, (i) *Poeonoma* Tams & Bowden 1953 (fig. 4), in which to place the species *P. serrata* (which Hampson had originally put in *Phragmatiphila)* and two other new species, and (ii) *Sciomesa* Tams & Bowden 1953 (fig. 5) to include three species that Hampson had placed in *Conicofrontia*. They
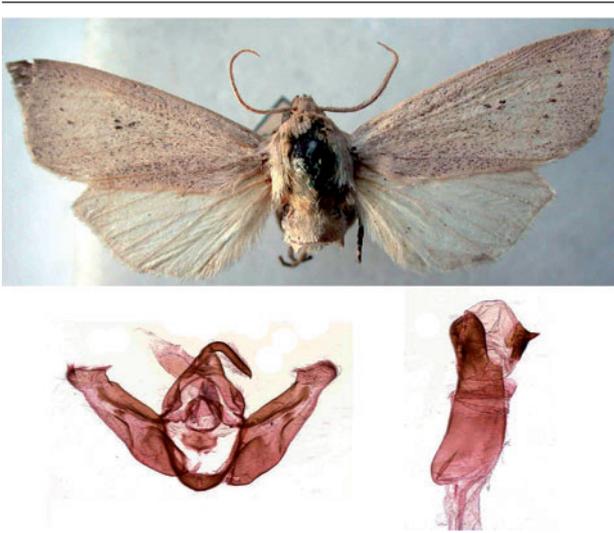
Figure 3
Speia vuteria (Stoll 1790) (A: adult; B: male genitalia; C: aedeagus).

Figure 5
Sciomesa mesophaea (Hampson 1910) (A: adult; B: male genitalia; C: aedeagus).

Figure 4
Poeonoma serrata (Hampson 1910) (A: adult.; B: male genitalia; C: aedeagus).

described also five new species of *Sesamia*. In 1956 Bowden described three new genera, *Carelis* Bowden 1956 (fig. 6), *Manga* Bowden 1956 and *Poecopa* Bowden 1956 (fig. 7), that were the last described genera in the group of African noctuid stem borers; he also described six new species. Not only was this work a masterpiece in systematics, but it was also the first time that wild host plants of many borers were recorded, and the papers by Tams & Bowden (1953) and Bowden (1956) remained until recently the most complete sources of information on the ecology of noctuid stem borers in natural habitats (Holloway 1998).

Figure 6
Carelis albula Bowden 1956 (A: adult; B: male genitalia; C: aedeagus).

The era of great expeditions

The observation of genitalia was a tremendous step forward and resulted in an unquestionable clarification of the taxonomy of the group. Where the only noctuid species ever found in maize, sugar cane, sorghum and rice crops in Africa was once *Sesamia vuteria*, it was now possible to distinguish *Sesamia calamistis*, *S. nonagrioides* and *Sesamia poephaga* Tams & Bowden (1953). But all problems were not solved, and the delimiting species remained a particularly difficult task, as was quickly proved. Thus Nye (1960) sank two species described by Tams and Bowden to the rank of subspecies: *Sesamia botanephaga* Tams & Bowden 1953 became a subspecies of *S. nonagrioides* and *Busseola segeta* Bowden 1956 a subspecies of *Busseola phaia* Bowden 1956. Apart from some isolated descriptions or revisions of this kind, most of the work on noctuid stem borers after Tams and Bowden was a consequence of localised expeditions: (i) The Ruwenzori expedition of 1952, which enabled Fletcher (1961) to describe 10 new species belonging to several of the genera already mentioned, and one new species he placed in the genus *Hygrostola* Warren 1913; (ii) The Ethiopian expeditions of Rougeot from 1976 to 1982 (Rougeot, 1984) which resulted in 18 new taxa described by Laporte (1984). (iii) The study of Madagascar noctuids by Viette (1967), which was done in the interim between the above two expeditions. Considerable work was also done by Berio (1973; 1975; 1976), who described 28 new species, mainly *Acrapex* (25), collected for the most part in Congo and Tanzania. During this period of 30 years following the papers by Tams and Bowden, the number of described African noctuid stem borer species was doubled (fig. 8). This intensive descriptive work was, however, highly biased towards two genera, *Acrapex* and *Sciomesa*, which accounted for 84% of the new species described. There was apparently a trend to place the new species in these two genera, although in several cases, there was doubt about the proper position. Thus *Sciomesa piscator* Fletcher 1961 was placed "provisionally" in

Figure 7
Poecopa mediopuncta Bowden 1956 (A: adult; B: male genitalia; C: aedeagus).

Number of described species
of African noctuid stem borers
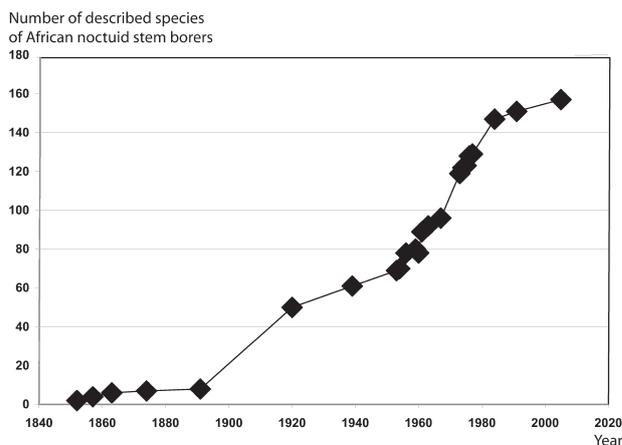
Figure 8
Progression of the number of described species of the *Sesamia sensu lato* group (Holloway 1998) of African noctuid stem borers since 1850.

this genus (Fletcher 1961), where it is still today. There was also a doubt about *Sciomesa biluma* Nye 1959, a species endemic to Madagascar and described by Nye (1959); the author found that this species did not easily fit in the mould of the described *Sciomesa* genus, but was reluctant to create a new genus for only one species. This intensive descriptive work was at that time more aimed at placing taxa in existing genera, even temporarily or inaccurately, rather than trying to improve the taxonomic relationships. Following this prolific time, the systematics of the group entered a new resting period. It finally became active again in Africa, where the history continues with the recent work by Krüger (2005) on the moths of Lesotho. This is the second contribution of an African author to the knowledge of African noctuid stem borers, and comes 66 years after Janse's book. Four new species of *Sesamia* and two new species of *Acrapex* are described.

Table 1. Geographic distribution of the *Sesamia sensu lato* group (Holloway 1998) of noctuid stem borers in the different regions of Africa.

Genus	Southern strictly[a]	Eastern[b] strictly	East or South	Central	Western strictly	West or East	Northern Sahara strictly	North or East	All regions sub-Sahara
Acrapex	15	37	1	12	4	1			
Busseola	2	1	1	1	3				1
Carelis					2				
Conicofrontia	2	1							
Hygrostola		1							
Manga		2		1	1				
Poecopa						1			
Poeonoma				2	2	1			
Sciomesa	2	16		2					
Sesamia	11	16	3	2	1		3	1	4
Speia		1							
Total	**32**	**74**	**6**	**20**	**13**	**3**	**3**	**1**	**5**
%	20.4	47.2	3.8	12.7	8.3	1.9	1.9	0.6	3.2

[a] "strictly" means the species is found only in this region
[b] eastern Africa includes Madagascar

Discussion

These taxonomic studies above enabled the description of 11 genera and 157 species of African noctuid stem borers of monocotyledonous plants: 70 *Acrapex*, nine *Busseola*, two *Carelis*, three *Conicofrontia*, one *Hygrostola*, four *Manga*, one *Poecopa,* five *Poeonoma*, 20 *Sciomesa*, 41 *Sesamia*, and one *Speia*. They reveal furthermore two features of these insects:

(i) From an applied point of view, few among these species are agricultural pests. Five are major pests, of which four are *Sesamia* species (*S. nonagrioides*, *S. calamistis*, *S. poephaga* and *Sesamia cretica* Lederer 1857) and one a *Busseola species*, *Busseola fusca* (Fuller 1901). Two other species appear to be of little economic importance: *Sciomesa biluma* is endemic to Madagascar where it is found on rice, maize and sorghum (Caresche & Breniere 1961; Breniere & Dubois 1965), and Manga basilinea Bowden 1956 is a pest of pearl millet, *Pennisetum glaucum* (L.) in western Africa (Bowden 1956; Harris 1962). This could be due to the host plant specialisation of most borers (Le Rü *et al.* 2006). However, several other species have been recently found in crops, particularly in maize, which suggests that they could become pests in future (Ong'amo *et al.* 2006).

(ii) From a biogeographical point of view, the borer distribution is highly biased: most borers species (71.4%) are known from eastern or southern Africa (tab. 1). Is this a consequence of poor prospecting in other parts of the continent, or is speciation favoured in the more various landscapes of these regions of Africa, or is this a combination of both factors? It is difficult to answer this question at the moment. Collecting has been very low in central Africa, and most of the data from West Africa originate from Bowden's work that was limited to Ghana, which suggests that there is still a lot to do in order to get a good picture of borer biodiversity in these regions.

Some remaining difficulties and future prospects

Although clarified by the work of all the above-mentioned taxonomists, the systematics of the group is still problematic in some cases, both at the species and genus level. Some remaining difficulties can be summed up as follows.

- For several species, only one sex is known. If it is a female (whose genitalia often have few distinctive characters), it can be difficult to decide in which genus to place it, and also to ascertain that it is not the female of a species already described elsewhere from a male specimen.

- Some species are so close, even with respect to their male genitalia, that it is not clear if they should be considered as different. For instance, Tams & Bowden said that further investigation might show *S. poephaga* and *Sesamia penniseti* Tams & Bowden 1953 to be races of one widely distributed species. Holloway (1998) in contrast suggests that possibly *S. poephaga* might be conspecific with *Sesamia epunctifera* Hampson 1902 on the one hand, and *S. penniseti* conspecific with *Sesamia poebora* Tams & Bowden 1953 on the other hand.

- How can we delimit the genera? Can we use genitalia to determine these limits? From the time of Tams and Bowden, a tendency to consider genitalia as the main criterion to group together species in genera became apparent, particularly for *Sciomesa* and *Acrapex*. However there are sometimes great morphological differences between the genitalia of species within a genus. For instance, Tams & Bowden (1953) observed such a case in the genitalial structure of *Sesamia* species, with two clear-cut groups, the *nonagrioides* and the *cretica* groups. These authors first thought of creating a new genus for the latter, but they then found that there were intermediate species, such as *S. jansei*, that have genitalia with features found in both groups; they therefore maintained one genus. But the problem is not solved in this particular case, however, since some other species placed in *Sesamia* have genitalia different from both groups, as for instance *Sesamia sciagrapha* Fletcher 1961 and *Sesamia sabulosa* Hampson 1910. Should we keep them in this genus, or is the genus paraphyletic, calling for the creation of a new one? Similar matters arise also for the other genera.

Nowadays a new tool is available that could be of considerable help in solving these questions: the use of molecular data. These techniques enable us, for instance, to attribute with certainty a female to a species. They can help in deciding whether or not

two taxa should be included in the same species. They can help in understanding the evolution of the group and therefore in delimiting monophyletic sets, thus enabling the definition of natural genera. Molecular techniques can also help dramatically in the identification of pre-imaginal stages.

While facing the deficit in taxonomists, and enthusiastic about the apparently unlimited possibilities in solving classification dilemmas through the use of molecular taxonomy, some authors have even suggested the creation of a bar-coding system based on a mitochondrial gene, Cytochrome Oxidase subunit 1 (Hebert *et al.* 2003), as a basis for the future description of Earth's animal biodiversity. This proposal has ardent promoters (Blaxter 2003, 2004; Tautz *et al.* 2003), but also strong opponents (Lipscomb *et al.* 2003; Lee 2004; Will & Rubinoff 2004). The use of molecular taxonomy alone, based on a single mitochondrial gene, has indeed serious flaws. For instance there can be introgressions of mitochondrial genes between close species that can hybridise: individuals of a species may have the mitochondrial genome of the other, which contradicts the results of morphological or nuclear data. A recent well known example is that of the African elephants (Roca *et al.* 2001; 2004); another instance in insects is the similarity of mitochondrial DNA between close species of *Drosophila* Fallen 1823 (Lachaise *et al.* 2000), which contrasts with the nuclear DNA, morphology and proven reproductive isolation, and which could be due to infection by *Wolbachia* endosymbiotic bacteria. Another problem is that copies of mitochondrial genes in the nuclear genome (called numts) sometimes occurred in the past; these can be amplified instead of the mitochondrial gene and result in wrong sequences (Richly & Leister 2004). It seems then more sensible, in our opinion, to promote a combined use of morphological and molecular observations to improve the knowledge of biodiversity. For instance, studies from DNA sequences (Moyal *in.lit.*) confirm that taxonomists were right when they were reluctant to place some species in the *Sciomesa* genus. This genus proved indeed to be paraphyletic at the molecular level. And the results of molecular analyses are congruent not only with adult morphology, but also with observations of the larval morphology, behaviour and host-plant preference (Le Rü *et al.* 2006). Therefore, just as the observation of genitalia was a great step that helped in clarifying the taxonomy based only on adult habitus, the use of molecular data in combination with morphological data (and also ecological and behavioural data, when available) should result in a new impulse in the systematics of African noctuid stem borers. Interest in such an approach for

the noctuid stem borer group is exemplified by the first studies on the *Manga* genus (Moyal & Le Rü 2006).

References

Berio E. 1973. Nuove species e generi di noctuidae africane e asiatiche e note sinonimiche. Parte II. *Annali del museo civico di storia naturale „Giacomo doria"* **79**: 126-171.

Berio E. 1975. Diagnosi di nuovi taxa di noctuidae del globo (Lepidoptera). *Annali del museo civico di storia naturale „Giacomo doria"* **80**: 321-339.

Berio E. 1976. Nuovi generi e specie di noctuidae dell'Africa equatoriale. *Annali del Museo civico di storia naturale „Giacomo doria"* **81**: 96-123.

Blaxter M.L. 2003. Counting angels with DNA. *Nature* **421**: 122-124.

Blaxter M.L. 2004. The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London B.* **359**: 669-679.

Bowden J. 1956. New species of African stem-boring Agrotidae (Lepidoptera). *Bulletin of Entomological Research* **47(3)**: 415-428.

Breniere J., Dubois J. 1965. Catalogue des insectes nuisibles aux cultures malgaches. *Institut de Recherches Agronomiques de Madagascar* **43**: 168pp.

Caresche L., Breniere J. 1961. Liste des insectes nuisibles aux plantes cultivées à Madagascar. *Institut de Recherches Agronomiques de Madagascar* **23**: 1-21.

Fletcher D.S. 1961. Noctuidae. **1(7)** p. 177-323 *in:* **Evans G. O., Fletcher D. S. (eds.),** *Ruwenzori expedition 1952.* British Museum (Natural History), William Clowes & Sons Ltd, London.

Guenée A. 1852. Noctuélites. **5** p. 64-111 *in:* **Boisduval J.B.A.D., Guenée A. (eds.),** *Histoire naturelle des insectes- Species général des Lépidoptères. Tome cinquième.* Librairie encyclopédique de Roret, Paris.

Hampson G.F. 1910. *Catalogue of the Noctuidae in the Collection of the British Museum.* Taylor & Francis, London, 552 p.

Harris K.M. 1962. Lepidopterous stem borers of cereals in Nigeria. *Bulletin of Entomological Research* **53(1):** 139-172.

Hebert, P. D. N., Cywinska A., Ball S.L., Dewaard J.R. 2003. Biological identifications through DNA barcodes. *Proceedings Royal Society London B* **270**: 313-321.

Holloway J. D. 1998. *Noctuidae.* p. 79-86 *in:* **Polaszek A. (ed.),** *African Cereal Stem Borers.* CABI, London.

Janse A.J.T. 1939. *The Moths of South Africa.* p. 307-435, *Volume 3. Cymatophoridae, Callidulidae and Noctuidae (partim).* E.P. & Commercial Printing Co. Ltd, Durban.

Krüger M. 2005. New genera and species of noctuid moths from Lesotho (Lepidoptera: Noctuoidea: Noctuidae). *African Entomology* **13(1):** 97-142.

Lachaise D., Harry M., Solignac M., Lemeunier F., Benassi V., Cariou M.-L. 2000. Evolutionary novelties in islands : *Drosophila santomea*, a new *melanogaster* sister species from São Tomé. *Proceedings of the Royal Society of London B.* **267**: 1487-1495.

Laporte B. 1984. Noctuidae. p. 12-51 *in:* **Rougeot P. C. (ed.),** *Missions entomologiques en Ethiopie 1976-1982. Mémoires du Museum national d'Histoire naturelle (Nouvelle série). Volmume 128, Fascicule II.* Museum National d'Histoire Naturelle, Paris.

Lee M.S.Y. 2004. The molecularisation of taxonomy. *Invertebrate Systematics* **18**: 1-6.

Le Rü B.P., Ong'amo G.O., Moyal P., Muchugu E., Ngala L., Musyoka B., Abdullah Z., Kauma-Matama T., Lada V.Y., Pallangyo B., Omwega C.O., Schulthess F., Calatayud P.-A., Silvain J.-F. 2006 Geographic distribution and host plant ranges of East African noctuid stem borers. *Annales de la Société entomologique de France (N.S.)* **42(3-4):** 353-361.

Lipscomb D., Platnick N., Wheeler Q. 2003. The intellectual content of taxonomy: a comment on DNA taxonomy. *Trends in Ecology and Evolution* **18(2):** 65-66.

Moyal P., Le Rü B. 2006. From population to species: morphological and molecular diversity in east African stem borer species of the genus *Manga* Bowden 1956 (Lepidoptera: Noctuidae). *Annales de la Société entomologique de France (N.S.)* **42(3-4):** 293-307.

Nye I.W.B. 1959. A new species of Amphypirinae from Madagascar. *Revue française d'Entomologie* **26**: 50-52.

Nye I.W.B. 1960. *The Insect Pests of Graminaceous Crops in East Africa*. Her Majesty's Stationery Office, London, 42 p.

Ong'amo G.O., Le Rü B.P., Dupas S., Moyal P., Calatayud P.-A., Silvain J.-F. 2006. Distribution, pest status and agro-climatic preferences of lepidopteran stemborers of maize in Kenya. *Annales de la Société Entomologique de France (N.S.)* **42(2):** 171-177.

Richly E., Leister D. 2004. Numts in sequenced eukaryotic genomes. *Molecular Biology and Evolution* **21(6):** 1081-1084.

Roca A.L., Georgiadis N., Pecon-Slattery J., O'Brien S.J. 2001. Genetic evidence for two species of elephant in Africa. *Science* **293**: 1473-1477.

Roca A.L., Georgiadis N., O'Brien S.J. 2004. Cytonuclear genomic dissociation in African Elephant species. *Nature Genetics* **37(1):** 96-100.

Rougeot P.C. 1984. Missions entomologiques en Ethiopie 1976-1982. *Mémoires du Muséum National d'Histoire Naturelle (N.S.)*, **128(2):** 1-93.

Tams W.H.T., Bowden J. 1953. A revision of the African species of *Sesamia* Guenée and related genera (Agrotidae-Lepidoptera). *Bulletin of Entomological Research* **43:** 645-679.

Tautz D., Arctander P., Minelli A., Thomas R.H., Vogler A.P. 2003. A plea for DNA taxonomy. *Trends in Ecology and Evolution* **18(2):** 70-74.

Thurau F. 1904. *Busseola sorghicida*, eine neue Ost-Afrikanische Noctuide. *Berliner Entomologische Zeitschrift* **49**: 55-58.

Viette P. 1967. *Insectes Lépidoptères Noctuidae Amphipyrinae (Part.) et Melicleptriinae. Faune de Madagascar*. ORSTOM-CNRS, Paris **20(2):** 394-417.

Will K.W., Rubinoff D. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* **20**: 47-55.

From population to species: morphological and molecular diversity in east African stem borer species of the genus *Manga* Bowden 1956 (Lepidoptera: Noctuidae)

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Abstract. Larvae of noctuid stem borers were collected from wild monocot plants in Eastern Africa, from Ethiopia to Mozambique, and reared to the adult stage. Three species of the African genus *Manga* Bowden 1956 (Lepidoptera: Noctuidae) were found, all restricted to host plants of the family Poaceae. *M. melanodonta* (Hampson) was collected in stems of *Panicum maximum* Jacquin, *Setaria megaphylla* (Steudel) Th. Durand & Schinz and *Setaria plicatilis* (Hochstetter) Engler; *M. nubifera* (Hampson) **stat. rev.**, and *M. fuliginosa* **n. sp.**, were both found only in stems of *P. maximum*. The second species was in the past sunk with *M. melanodonta* as a synonym, but the present study shows its validity. Descriptions are given of the new species as well as of features not yet described of known species (female habitus and male and female genitalia of *M. melanodonta* and M. nubifera) and of the intraspecific morphological variation observed in the male genitalia. Larval morphology and life habits are described. Pictures of the adults and genitalia of the other species of the genus are provided except for *M. bisignata* Laporte that is sunk with *Busseola quadrata* Bowden as a synonym (**n. syn.**). The molecular diversity of the collected species was studied using the mitochondrial gene Cytochrome *b*. A complex history of successive fragmentation events was revealed. The combination of three forces appeared to have shaped this diversity: the main paleo-climatic events (successive dry and humid periods), the geological barriers, particularly the Rift Valley, and specialization on new host plants. A molecular clock proved to be acceptable for all clades except for the species that first diverged, *Manga fuliginosa*. The dates of the major paleo-climatic events of the last 5 million years appeared to correspond to the observed divergence events when using an evolutionary rate of 1.15% per million years, with a correction for *M. fuliginosa*. Isolation by the Rift Valley favoured diversification in some instances, and the adaptation of *Manga melanodonta* to new host plants enabled the colonization of humid environments. A scenario for the evolution of the group is proposed, from its origin in Austral Africa about 5 million years ago and its northward expansion, until the recent migrations of *Manga nubifera* during the past million years.

Résumé. De la population à l'espèce : Diversité morphologique et moléculaire des foreurs de graminées d'Afrique de l'Est du genre *Manga* Bowden 1956 (Lepidoptera : Noctuidae). Des larves de noctuelles foreuses ont été récoltées dans les tiges de monocotylédones sauvages en Afrique de l'est, de l'Éthiopie au Mozambique, et élevées jusqu'au stade adulte. Trois espèces du genre africain *Manga* Bowden 1956 (Lepidoptera : Noctuidae) ont été trouvées, dans des plantes hôtes appartenant uniquement à la famille des Poaceae. *M. melanodonta* (Hampson) a été récoltée dans des tiges de *Panicum maximum* Jacquin, *Setaria megaphylla* (Steudel) Th. Durand & Schinz et *Setaria plicatilis* (Hochstetter) Engler; *M. nubifera* (Hampson) **stat. rev.** et *M. fuliginosa* **n. sp.**, ont toutes deux été récoltées seulement dans des tiges de *P. maximum*. La deuxième espèce avait été dans le passé mise en synonymie avec *M. melanodonta*, mais la présente étude montre sa validité en tant qu'espèce. Des descriptions sont données de la nouvelle espèce ainsi que de caractères non encore décrits d'espèces connues (habitus femelle et genitalia mâles et femelles de *M. melanodonta* et *M. nubifera*) et de la variabilité intraspécifique observée sur les genitalia mâles. La morphologie et les traits de vie des larves sont décrits. Des photos des adultes et genitalia des autres espèces du genre sont fournies excepté pour l'espèce *M. bisignata* Laporte, qui est mise en synonymie avec *Busseola quadrata* Bowden (**n. syn.**). La diversité moléculaire des espèces récoltées a été étudiée au niveau du gène mitochondrial Cytochrome *b*. Une histoire complexe, faite d'événements de fragmentation successifs, a été mise en évidence. La combinaison de trois forces semble avoir façonné cette diversité : les événements paléo-climatiques majeurs (succession de périodes sèches et humides), les barrières géologiques, en particulier la Vallée du Rift, et la spécialisation sur de nouvelles plantes-hôtes. Il est apparu que l'hypothèse d'une horloge moléculaire était acceptable pour tous les clades à l'exception de l'espèce ayant divergé le plus anciennement, *Manga fuliginosa*. Les dates des événements paléo-climatiques majeurs des 5 derniers millions d'années sont apparues correspondre aux événements de divergence observés si l'on adopte un taux d'évolution de 1,15% par million d'année, avec une

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correction pour *M. fuliginosa*. L'isolement par la Vallée du Rift a parfois favorisé la diversification, et l'adaptation à de nouvelles plantes-hôtes de *Manga melanodonta* a permis la colonisation de biotopes humides. Un scénario de l'évolution du groupe est proposé, depuis son origine en Afrique australe il y a environ 5 millions d'années et son expansion vers le nord, jusqu'aux récentes migrations de *Manga nubifera* durant le dernier million d'années.

Keywords: Graminaceous stem borer, *Manga*, Molecular clock, Noctuidae, Poaceae.

Noctuid stem borers of graminaceous plants are important pests of crops in Africa where severe yield losses were reported from countries South of Sahara, in Southern (Van Den Berg *et al.* 2001), Western (Moyal 1998), and Eastern Africa (Khan *et al.* 2001; De Groote 2002) as well as from countries North of Sahara (Moyal *et al.* 2002). In order to get a better insight into the ecology and the way of controlling these pests, studies in wild environments have been recommended for a long time (Bowden 1976). Understanding the infestation dynamics, the possibilities of survival of an introduced parasitoid and estimating the risk of shift of a species from wild host plants to cultivated ones need to extend the studies of these insects outside of the crops. A first approach of this kind was carried out in Eastern Africa (Polaszek & Khan 2000) and in Western and Central Africa (Schulthess *et al.* 1997), which led to a better knowledge of wild host plants, mainly for known pests. Studying borer populations in natural landscapes requires the identification of species, which may be difficult, particularly in noctuid borers, because of the great morphological similarity between species and some intra-specific variability (Tams & Bowden 1953; Holloway 1998). So the combined use of morphological and molecular data has been promoted as a powerful tool to get a better insight into the taxonomic relationships in noctuid stem borers (Moyal 2006). Molecular studies can furthermore reveal the recent history of taxa. This approach was used in the present paper, which has the following purposes: (i) to clarify the taxonomy of the small African borer genus *Manga* Bowden 1956 (Lepidoptera: Noctuidae), that includes until now four species (Poole 1989) among which *M. basilinea* Bowden 1956, a pest of pearl millet, *Pennisetum glaucum* (L.) in Western Africa (Bowden 1956; Harris 1962); (ii) to show the morphological variability in the three East African species collected; (iii) to show the phylogenetic relationships and the molecular diversity of these species; (iv) to propose a scenario for the causes and the timing of their evolution.

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Material and Methods

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Morphological study

Stem borer larvae were collected from wild and cultivated host plants in several countries of Eastern and Southern Africa:
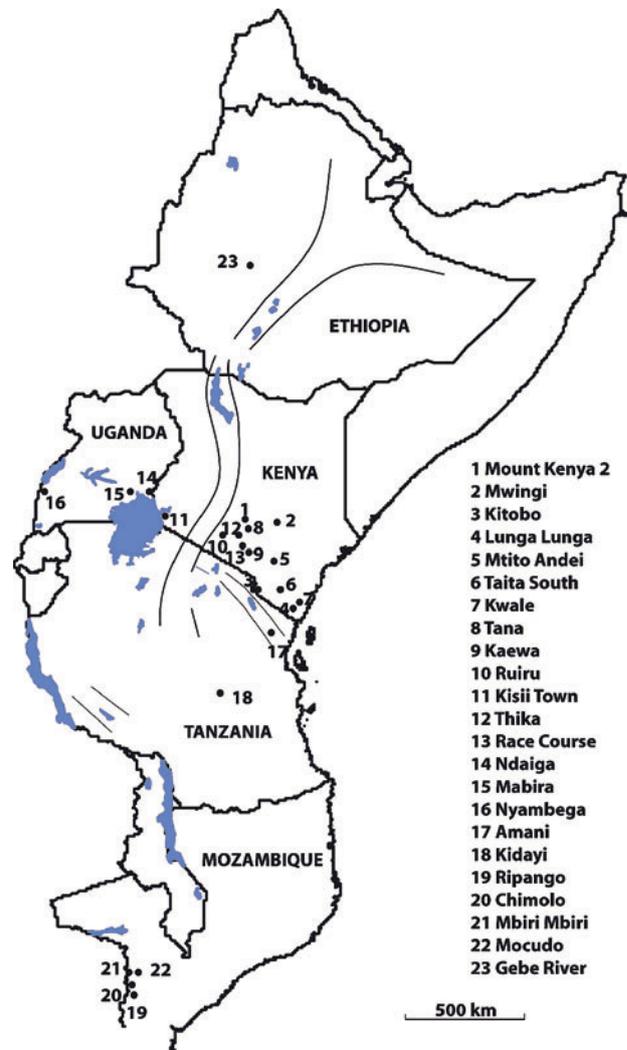

Figure 1
Localities sampled.

Eritrea, Ethiopia, Uganda, Kenya, Tanzania, Mozambique, Zambia, Rwanda and Zimbabwe. They were then reared on artificial diet (Onyango & Ochieng'Odero 1994) until pupation and emergence of adults. Some adults were kept in absolute alcohol for molecular analyses, others were kept dry and prepared as vouchers for museum collections and also used for molecular studies. Genitalia were dissected after a short stay in boiling potash 10% bath, and then mounted on slides in Euparal. The type of a new species collected is deposited in

the Museum National d'Histoire Naturelle (MNHN) in Paris, France. Figure 1 shows the location of the sampling sites and table 1 lists the precise geographic position of the sites together with relevant host plant records.

Molecular study

Most of the mitochondrial gene Cytochrome *b* (940 out of a total of 1134 nucleotides) was sequenced from 76 specimens. This gene is widely used in molecular studies in vertebrates and proved to be interesting in insects, particularly at the infra generic level (Simmons & Weller 2001).Total DNA was extracted using a Qiagen DNeasy tissue kit (Qiagen GmbH, Germany). The Cytochrome *b* gene was amplified by Polymerase Chain Reaction (PCR) using the successive steps: initial denaturation for 5 min at 92 °C; 39 cycles of denaturation for 1 min at 92 °C, annealing for 1.30 min at 46 °C, extension for 1.30 min at 72 °C; final extension for 5 min at 72 °C. The reaction mixture contained 3 mM $MgCl_2$, 0.4 µM primers, 0.24 µM dNTPs, 2 U of Promega *Taq* polymerase and 100 ng of DNA per 50 µl of reaction mixture. The primers used were CP1 (5'-GATGATGAAATTTTGGATC-3') [modified from Harry *et al.* (1998)] and TRs (5'-TCTATCTTATGTTTTCAAAAG-3') (Simon *et al.* 1994). The PCR product was then purified using the Qiagen QIAquick PCR purification kit (Qiagen GmbH, Germany). Sequencing reactions were carried out using the Sanger dideoxy method (Sanger *et al.* 1977), and finally sequences were run and detected on an ABI 377 automated sequencer. A portion of the mitochondrial gene cox1 (Cytochrome c Oxidase, subunit 1) (894 nt) was also sequenced for a limited number of insects in order to study the possibility of dating the divergence events (see below). The primers used were Ron (5'-GGATCACCTGATATAGCATTCCC-3') and Hobbes (5'-AAATGTTGNGGRAAAAATGTTA-3') (Monteiro & Pierce 2001) and the PCR protocol was the same as for Cytochrome *b*.

Phylogenetic analysis

The obtained sequences were aligned using Multalin (Corpet 1988). The best substitution model was selected with Modeltest ver 3.7 (Posada & Crandall 1998) in combination with Paup 4.0b10 (Swofford 2003) using the Akaike information criterion (Posada & Buckley 2004). The values of the selected parameters were then used to perform the phylogenetic analysis with Phyml software (Guindon & Gascuel 2003). The algorithm used in this software enables a fast estimate of the best phylogenetic tree using the maximum-likelihood principle, thanks to the simultaneous adjustment of tree topology and branch lengths, using the nearest neighbour interchange as branch swapping method. Except the selected parameters, the default options proposed by Phyml were used, with a starting tree built by BIONJ (Gascuel 1997). This analysis was followed by non parametric bootstrapping of 700 replicates. Three species belonging to closely related borer genera were chosen as outgroups: *Busseola fusca* (Fuller 1901), *Sesamia calamistis* Hampson 1910 and *Sciomesa nyei* Fletcher 1961. The phylogenetic tree was then displayed as a graphic in Treeview ver 1.6.6 (Page 1996). Genetic distance and divergence dates were calculated with Mega 3.1 (Kumar *et al.* 2004). Diversity at the population level was studied only in *M. nubifera* (49 sequenced individuals). Haplotype networks were constructed according to the method of Templeton *et al.* (1992) with TCS version 1.18 (Clement *et al.* 2000). Arlequin software (Schneider *et al.* 2000) was used to calculate diversity parameters (gene diversity, pairwise differences and nucleotide

diversity) and migrant numbers between populations using the Slatkin's method (1991).

To understand the role of paleo-climatic events on the diversification of the species, it is necessary to be able to date the divergence events. Estimation of the rate of evolution in insects is difficult because of the lack of fossils in many orders. Two studies have attempted to solve this question, using mitochondrial DNA. These two methods were compared to estimate the divergence dates between the observed clades of *Manga*. The first one, proposed by Brower (1994), compared the evolution rate of recent species (whose divergence occurred less than 3.5 million years ago) belonging to several orders, using several methods of calibration of the evolutionary rate (e.g., fossil dating when available and biogeography). Different methods of studying mitochondrial diversity were used, depending on the available data (Restriction enzymes sites and sequences of Cytochrome c Oxidase subunit 1). This study indicated that the evolution rate was rather constant (i.e. a molecular clock is acceptable) whatever the method used and the insect order considered. The evolutionary rate of mitochondrial DNA was then estimated to be 1.1-1.2% per million years, i.e. an average pairwise divergence of 2.3%. The second method was used by Gaunt & Miles (2002) in order to date all the important events of the insect evolution, and then much older divergences. The use of slowly evolving sites was then necessary. Their study, based on cox1 gene, examined the evolution of the second codon position and aminoacids. They showed that a molecular clock was acceptable in the first case but that local molecular clocks had to be fitted for protein evolution. These two methods may result in large differences between estimates of divergence events, for instance an evolution rate about 6 times slower in the second method in the case of Papilionidae (Zakharov *et al.* 2004). Therefore one needs to estimate which method is most appropriate to get a correct estimate of speciation events. The hypothesis of a molecular clock was tested with Mega 3.1, using the relative rate test of Tajima (1993).

Results

Specimens of *Manga* Bowden 1956 were found in five of the surveyed countries: Ethiopia, Kenya, Tanzania, Uganda and Mozambique and in only three host plants: *Panicum maximum* Jacquin 1781, *Setaria megaphylla* (Steudel) Th. Durand & Schinz 1894 and *Setaria plicatilis* (Hochstetter) Engler 1891 (Poaceae). Three species were collected: *Manga melanodonta* (Hampson 1910) and two other species, i.e. one new species (described below as *M. fuliginosa* **n. sp**) and a species corresponding to *M. nubifera* (Hampson 1910) that was however sunk with *M. melanodonta* as synonym by Fletcher (1961). This study showed that it was a true species and its status is therefore re-appreciated. Indeed, the types of both *M. melanodonta* and *M. nubifera*, examined at the Natural History Museum of London, showed slight morphological differences, particularly in the male genitalia (figs. 3a & 5a). These differences, as well as differences in the habitus, were also observed in the numerous specimens collected, showing that both taxa are morphologically distinct. Studies at the molecular level confirmed that
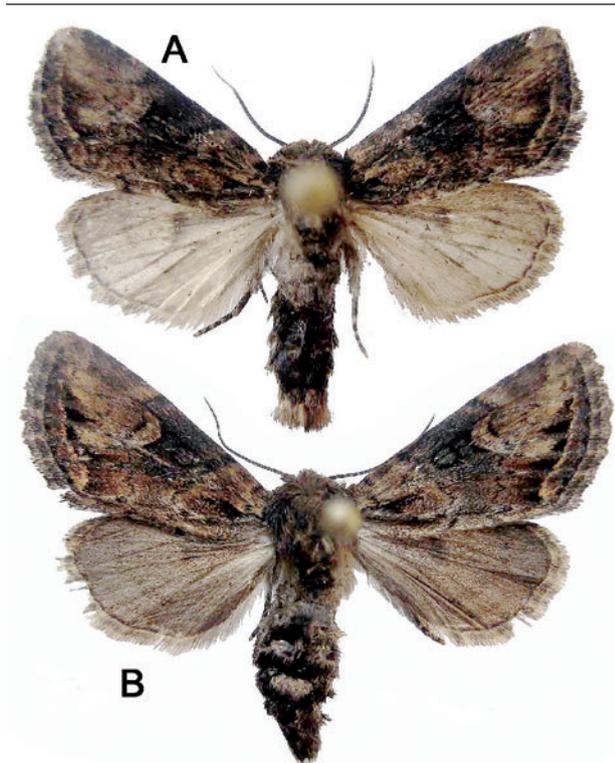
Figure 2
Adults of *Manga melanodonta*. A. Male; B. Female.

both groups diverged long ago. *Manga nubifera* and *M. fuliginosa* were found only in stems of *P. maximum*, whereas *M. melanodonta* was found in the three plants. Sampling in localities where the three borer species were present, e.g. Ripango, showed that all the specimens found in *S. megaphylla* belonged to *M. melanodonta*; moreover the other species were absent in places where only *S. megaphylla* or *S. plicatilis* were present. *M. melanodonta* was found mainly in humid forests such as the Guineo-Congolian vegetation mosaics, whereas *M. nubifera* was located mostly in dry forests or in forest galleries in dry vegetation mosaics (Zambesian miombo and Somalia-Masai mosaics). Too few specimens of *M. fuliginosa* were collected to enable conclusions on its ecological preferences.

Morphological study

Descriptions are given below of the new species, for which only two males were collected. Only the habitus of the male of *M. melanodonta* and *M. nubifera* were known until now and described by Hampson (1910). The male genitalia as well as the females were not known and are described here. Descriptions of larval morphology and ecology, that were unknown until now, are also given.
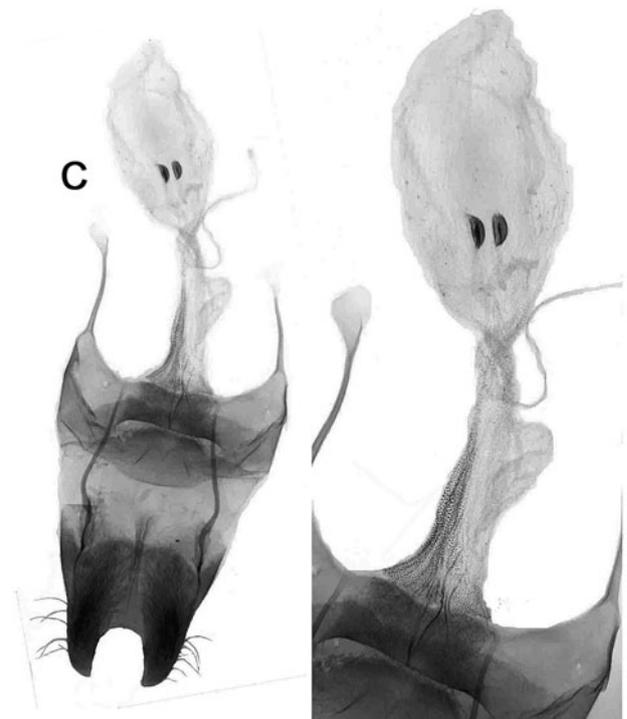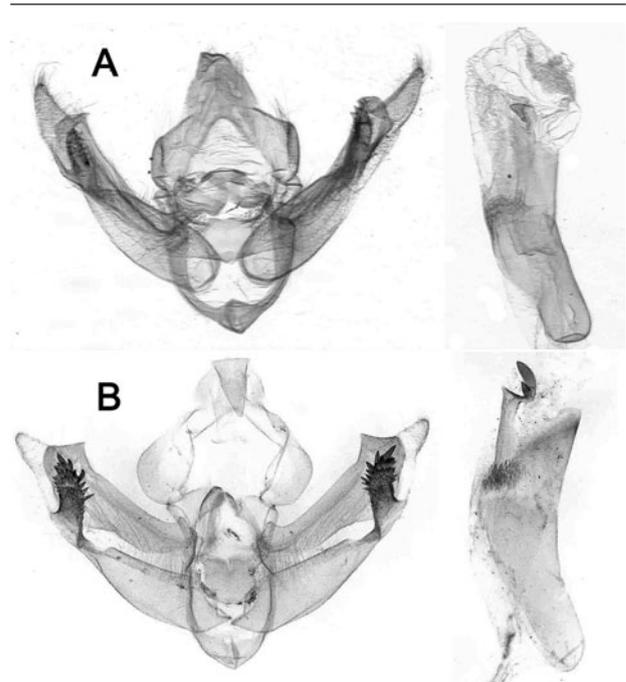

Figure 3
Genitalia of *Manga melanodonta*. A. Male, holotype; B. Male, specimen from Mozambique (Chimolo); C. Female (Uganda, Mabira).

Table 1. Sampling sites and host-plants (S: *Setaria megaphylla* or *S. plicatilis*; P: *Panicum maximum*) of the *Manga* species collected and sequenced.

Country	Site	Latitude	Longitude	*Manga* species	Host-plant
Kenya	Mount Kenya	S 00°43'02"	E 37°16'02"	*M. nubifera*	P
	Mwingi	S 00°33'24"	E 38°03'24"	*M. nubifera*	P
	Kitobo	S 03°15'54"	E 37°22'12"	*M. nubifera*	P
	Lunga Lunga	S 04°19'30"	E 39°04'42"	*M. nubifera*	P
	Mtito Andei	S 02°24'23"	E 38°07'02"	*M. nubifera*	P
	Taita South	S 03°17'51"	E 38°11'12"	*M. nubifera*	P
				M. melanodonta	P
	Kwale	S 04°05'18"	E 39°16'11"	*M. nubifera*	P
	Tana	S 00°47'21"	E 37°15'55"	*M. nubifera*	P
	Race Course	S 01°31'33"	E 37°14'38"	*M. melanodonta*	P
	Ruiru	S 01°03'26"	E 36°32'53"	*M. melanodonta*	P
	Kisii Town	S 00°23'55"	E 34°26'23"	*M. melanodonta*	S
	Thicka	S 01°00'46"	E 37°04'17"	*M. melanodonta*	P
	Kaewa	S 01°26'32"	E 37°18'32"	*M. melanodonta*	P
Uganda	Ndaiga	N 00°21'09"	E 34°01'58"	*M. nubifera*	P
	Mabira	N 00°26'27"	E 33°10'21"	*M. melanodonta*	P
	Nyambega	N 00°30'05"	E 30°07'34"	*M. melanodonta*	P
Tanzania	Amani	S 05°03'39"	E 38°24'18"	*M. nubifera*	P
	Kidayi	S 07°20'52"	E 36°28'30"	*M. nubifera*	P
Mozambique	Ripango	S 19°15'46"	E 33°10'37"	*M. nubifera*	P
				M. melanodonta	S
				M. fuliginosa	P
	Chimolo	S 19°02'43"	E 33°21'27"	*M. melanodonta*	S
	Mbiri Mbiri	S 18°31'01"	E 32°26'09"	*M. melanodonta*	P
	Mocudo	S 18°30'36"	E 32°27'07"	*M. melanodonta*	S
Ethiopia	Gebe River	N 08°08'06"	E 37°20'43"	*M. nubifera*	P

Figure 4
Adults of *Manga nubifera*. A. Male; B. Female.

Manga melanodonta (Hampson 1910)

(Figs 2 & 3)

Male genitalia. Tegumen broadly triangular, with medium-sized rounded peniculi. Vinculum narrow, forming a moderate saccus, like a small bulge of vinculum. Valve narrow, elongate; costa broad and slightly sclerotized with a well-developed ridge-like expansion. Sacculus narrow, broadest at base, gradually tapering distally, without produced clavus; clasper well developed, heavily spinose, straight and of rather constant width from its basis to apex; cucullus broad, fairly triangular with a more or less sharp apex; clasper and cucullus showing some geographic variability (see further); juxta simple, broad, shield-shaped. Uncus short and broad. Aedeagus short, stout, slightly dilated at apex. Apex extending ventrally into a recurved band ending in one or several spines (fig. 3 where the band was everted). Vesica membranous lacking cornuti. Manica membranous with lateral hair tufts.

Female. Similar to the male described in Hampson (1910) except for the ground colour of hindwing which is brown-grey, so that the discal spot is inconspicuous. Ground colour of the wings is generally darker than in the male, rendering the features less easy to distinguish. The female is larger than the male (wingspan: 26-27 mm versus 23-26 mm for the male).

Female genitalia. Both pairs of apophyses long and slender, with little sclerotized spatulate tips. Antrum ovoid, ostium bursae with anterior lip indented and convex in its central part. Sternum A8 slightly sclerotized only anteriorely to ostium bursae. Ductus bursae broad, with a faint punctuated sclerotisation. Bursa copulatrix elongate, elliptic, the length about twice the width, with paired signa; both signa located at approximately one third to one half of the bursa length from the junction with ductus; signa elongate, elliptic or drop-shaped, the length about twice the width, and divided into two parts by a longitudinal midline, generally well conspicuous. Ductus seminalis from junction of bursa and ductus bursae. Ovipositor lobes narrowly triangular, with the apex slightly curved inwards, bearing numerous long setae on the dorsal surface and shorter and sparser setae on the ventral side. The apex of the lobes bears several stout setae (in most cases between five and ten).

Material examined. Cf. Tab. 1.

Manga nubifera (Hampson 1910) stat. rev.

(Figs 4 & 5)

Male genitalia. Similar to *M. melanodonta* but with the following differences: Saccus better defined, narrower and with a roughly quadrangular shape. Valvae with costal sclerotization narrower; cucullus long, slender and with rounded apex; clasper heavily spinose, thick in its basal part, and terminating with sharp narrowing apex and angled towards the cucullus.

Female. Similar to the male described by Hampson (1910) except for the ground colour of the hindwing which is brown-grey, and where the discal spot is inconspicuous. The female is on average larger than the male (wingspan: 25-29 mm versus 24-28 mm for the male).

**Female genitalia.** As for *M. melanodonta*.

Material examined. Cf. Tab. 1.
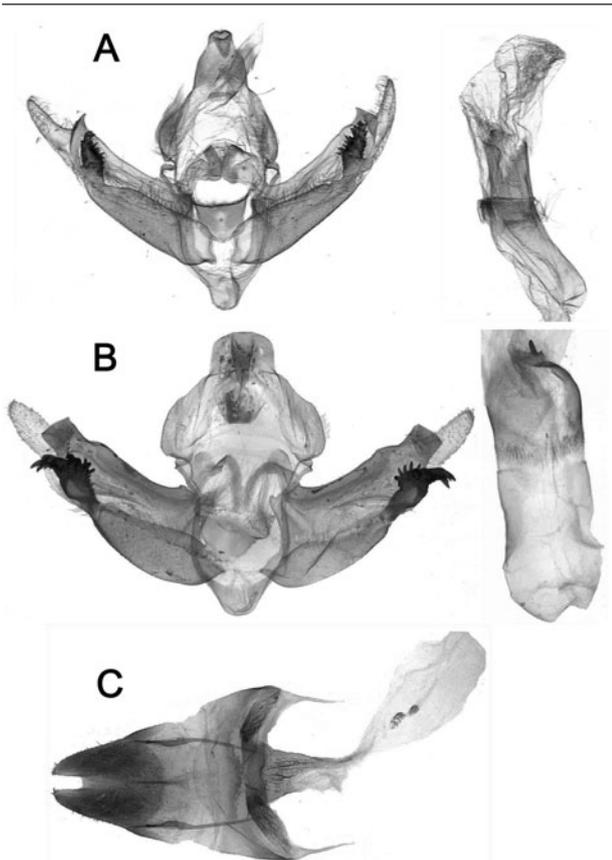
Figure 5
Genitalia of *Manga nubifera*. A. male, holotype; B. male, specimen from Ethiopia (Gebe River); C. female (Kenya, Kitobo).

Figure 6
Manga fuliginosa. A. Male adult; B. Male genitalia.

Manga fuliginosa n. sp.
(Fig. 6)

Material examined. Holotype: ♂, Mozambique, Ripango (19°15'46" S, 33°10'37" E), IV.2005, ex larva (in stem of *P. maximum*), B. Le Rü leg., gen. prep. MP1, MNHN, Paris.

Paratype. ♂, same data as above, gen. prep. MP2, in coll. P. Moyal.

Male. Antennae filiform with the dorsal surface covered with white scales. Head, palpi and thorax dark brown mixed with yellow brown; tarsi brown with slight pale rings; abdomen grey. Fore wing grey before antemedial with a short black basal streak; dark-grey between antemedial and postmedial lines except for a grey-brownish median streak. Claviform and orbicular absent; reniform faint. Subterminal line faint; subapical black spot fairly diffuse. Colouration between postmedial and subterminal lines mainly grey mixed with fuscous brown in the anal half, and dark grey between the subterminal and terminal lines. Hindwing pale grey with a faint discal spot and postmedial line. Wingspan: 20 mm

Male genitalia. Similar to *M. melanodonta* but with the following differences: saccus like a broad bulge of vinculum. Valvae with broad and triangular cucullus with a rounded


Figure 7
Larva of *Manga nubifera*. A. Young; B. Mature.

apex and strongly convex sacculus; clasper curved inwards, little spinose, with only some strong spines at the apex. The sclerotized band at the apex of the aedeagus with curved sharp spines.

Female. Unknown.

Larval morphology (fig. 7) and preimaginal life habits. The larvae of the three species were morphologically indistinguishable. Their main traits varied from the first to late instars. Young larva, from the first to the third instar (fig. 7A): head smooth and black, prothoracic shield, pinacula and anal plate dark brown, body with variable ground colour from white to pale pink with four characteristic black spots, two dorsal and two lateral, on each of the last three abdominal segments.

Mature larva (fig. 7B): length 30-35 mm, width 3.5 mm. Head smooth and brown, prothoracic shield pale-brown; body with ground colour dark grey, dorsally suffused with pale grey, pinacula and anal plate dark-brown.

Like other noctuid stem borers, eggs are laid between the leaf sheath and stem in batches of up to 50 eggs at the bottom of the spike. After hatching, larvae do not feed on leaves and penetrate the spike where they remain gregarious during the first two-three instars. More than 50-60 second instar larvae are commonly found in the same spike. Usually the apical part of the spike dries and, under dry weather conditions, the second and the third instar larvae remain quiescent until the beginning of the next rainy season, when they become active and disperse onto the stems and leaves of neighbouring plants.

Molecular study

Phylogenetic analysis, geographic distribution and host-plant

The sequences were submitted to GenBank (accession numbers DQ536363-DQ536396 and DQ628527-DQ628539). The best model of DNA evolution selected was HKY (Hasegawa *et al.* 1985) with a proportion of invariant sites of 0.64 and a Gamma distribution with a shape parameter of 1.53. The maximum likelihood phylogenetic tree obtained (fig. 8) shows different clades strongly supported by high bootstrap values. The first divergence event resulted in two species, *M. fuliginosa* and the ancestor of *M. melanodonta* and *M. nubifera*, which divided then into these two species. *M. nubifera* divided then into two clades, one of them with a geographic distribution limited to the Kenyan eastern part of the Rift Valley (MN1) (fig. 9), the second one (MN2) stretching from Ethiopia to Mozambique, limited to the west of the Rift Valley in Ethiopia, Uganda and Kenya, except, in the latter country, in the southern locality of Kitobo Forest. The population of Uganda (Ndaiga) appeared to be the sister group of all other populations in MN2. In *M. melanodonta*, a first division resulted in a Mozambique group (MOZ) and the ancestor of a northern group (Uganda-Kenya), which then split into two clades, one in Uganda (U) and one in Kenya
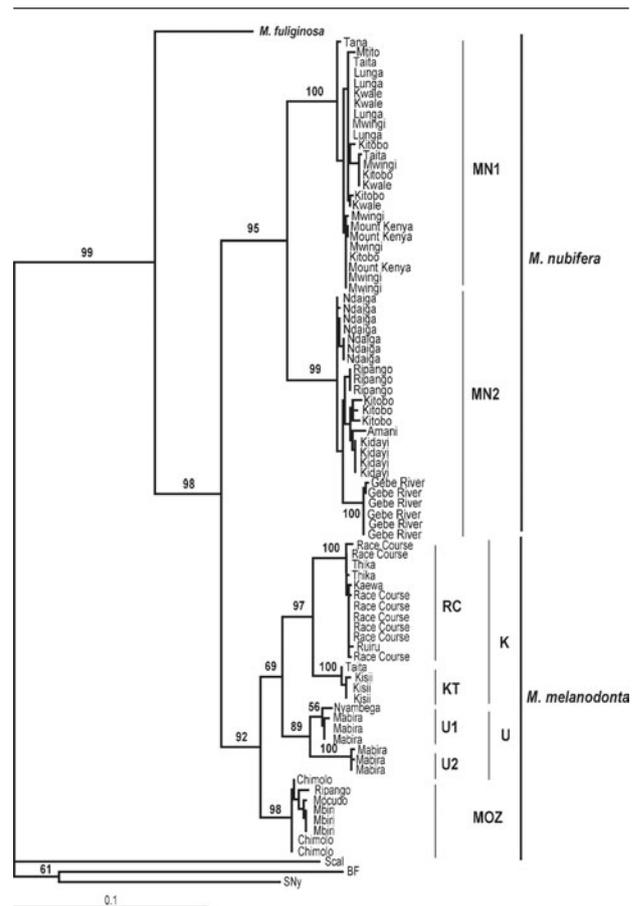

Figure 8
ML phylogenetic tree with the bootstrap values over 50%. Outgroups: SNy: *Sciomesa nyei*, BF: *Busseola fusca*; Scal: *Sesamia calamistis.*

(K) (fig.10). Within these clades a further division occurred: in the Kenyan group, two clades diverged, RC (for Race Course) and KT (for Kissii Town) east and west of the Rift Valley, respectively. In Uganda two different clades (U1 and U2) were found in the same localities. Inside these clades, the genetic distance between specimens was low, between 0 and 0.5%, typical of population variations. The genetic distances between clades were similar in several cases: 0.0241 (std err 0.0045) between U1 and U2, 0.0290 (std err 0.0054) between RC and KT; 0.0498 (std err 0.0064) between MN1 and MN2; and 0.04325 (std err 0.0062) between U and K. The p-distance between both species was 0.06635 (std err 0.0070).

Dating the divergence events

The method used by Gaunt & Miles (2002) appeared to be difficult to use in the present study. Indeed, no variation was observed between *Manga* species for the
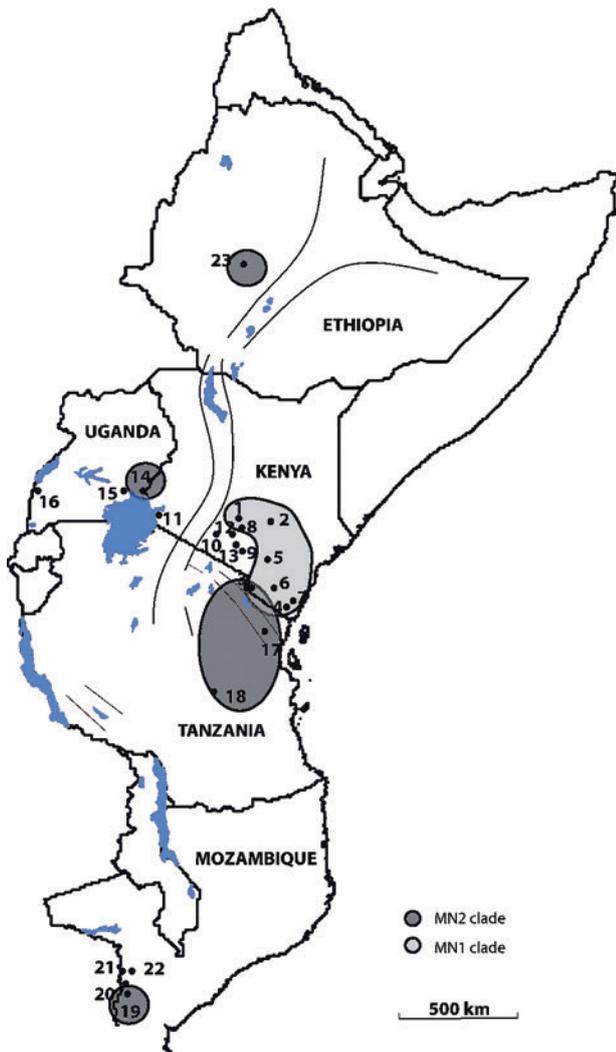
Figure 9
Geographic distribution of the clades of *Manga nubifera*.
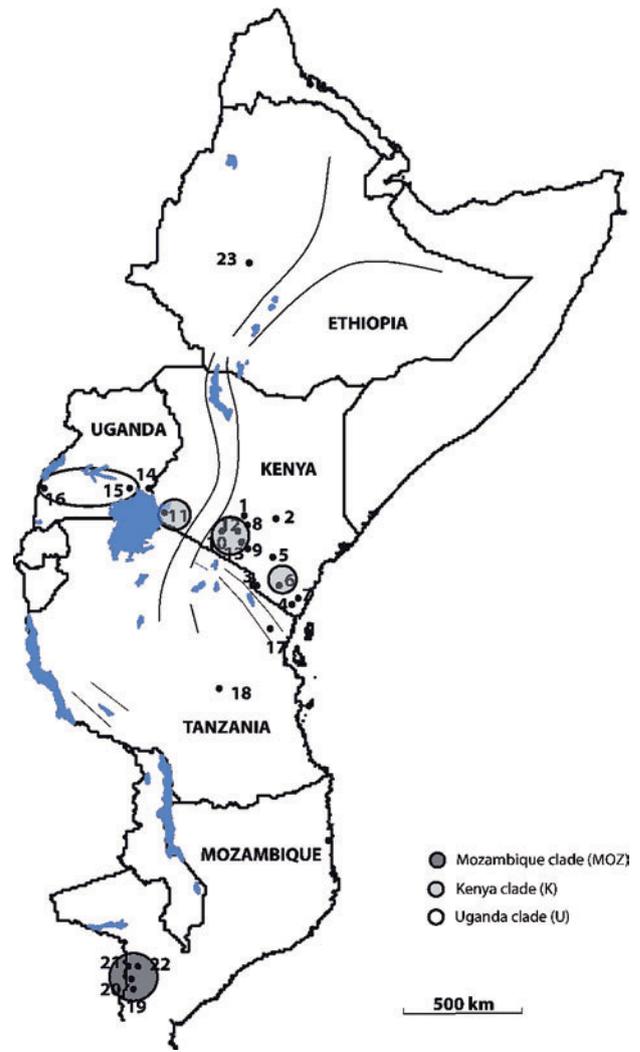
Figure 10
Geographic distribution of the clades of *Manga melanodonta*.

second codon in the sequenced part of the cox1 gene. Also, among the 298 amino acids, only one mutation was observed, which separates *M. nubifera* from the other *Manga* species. *M. fuliginosa*, which is the sister group of the other two *Manga* species, did not differ from *M. melanodonta* for the amino acid composition. Dating divergence using Brower's method, assuming an evolutionary rate of 1.15% by million years, was done for the different nodes. Most of the clades have separated distribution areas, which suggests that their evolution was independent. The results of the Tajima's relative rate test indicated that the hypothesis of a molecular clock could not be rejected in all cases except for *M. fuliginosa*. The plot of transitions versus transversions (fig. 11) showed indeed an increase from

1 to 12 transversions, but beyond the transition level seems rather constant, which indicates an apparent saturation that corresponds to the comparisons with *M. fuliginosa*. The regression line fitted was highly significant for the first part (without *M. fuliginosa*) ($R^2 = 0.592$; $F = 736.0$, $df = 1$ and 507) whereas there was no significant relationship and the slope was not different from zero for the distances including *M. fuliginosa* ($R^2 = 0.0024$; $F = 0.0081$ $df = 1$ and 33). Since the hypothesis of a molecular clock was acceptable for all the clades for which the regression line was significant, this linear relationship between transitions and transversions can be considered as the expression of this constant evolutionary rate. The actual divergence between *M. fuliginosa* and the other

Table 2. Datation of divergence events assuming an evolutionary rate of 1.15% per million year (MF = *M. fuliginosa*; MM = *M. melanodonta*; MN = *M. nubifera*), with correction for MF (see text).

Node	Divergence date in Million years
MF vs Ancestor MM-MN	4.61
MM vs MN	2.91
MN1 vs MN2	1.99
MOZ vs U vs K	1.86
RC vs KT	1.34
U1 vs U2	1.02

clades if no saturation had occurred should then be correctly estimated through the extrapolation of the regression line. A corrected pairwise distance between *M. fuliginosa* and the other clades was then estimated for the average transversion distance observed (17.34). This yielded a total pairwise distance of 100.07 mutations, and then an estimated divergence date of 4.61 million years (Myr) (tab. 2).

Intraspecific diversity in *M. nubifera*

The intraspecific sampling of *M. nubifera* enabled a first comparison between the population diversity of two clades differing in their distribution area: the first one, limited to Kenya east of the Rift Valley (MN1), and the second one largely distributed from Ethiopia to Mozambique, and mainly west of the Rift Valley (MN2). The haplotype network of MN1 is characterized by closely related haplotypes (fig. 12A) and the presence of several different haplotypes in the localities. The haplotype network of MN2 (fig. 12B) is much more extended, with particularly the population of Ethiopia (Gebe River) very distant from the others
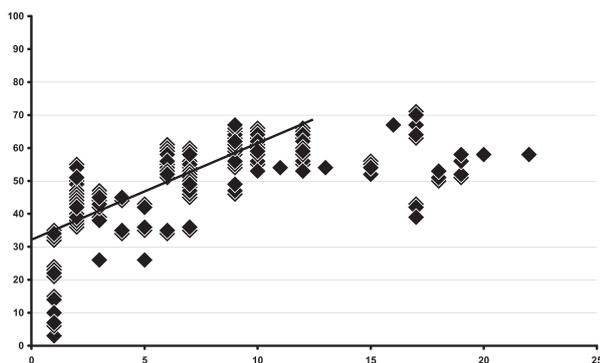

Figure 11
Plot of the pairwise number of transitions versus the pairwise number of transversions, with the significant regression line for the data without *Manga fuliginosa* (see text).

(12 mutations from the closest populations) and also an haplotype from Tanzania (Amani) rather far from the centre of the network. This centre includes populations that were geographically close (Uganda, Centre Tanzania and South Kenya) but also the very geographically distant population of Mozambique (2257 km between Ndaiga and Ripango vs 934 km between Ndaiga and Gebe River).

The gene diversity is high and similar in both clades (tab. 3), but MN2 is much more diverse at the nucleotide level, due particularly to the Ethiopian population. The gene flow between populations appear to be much higher in MN1 than in MN2, where all populations seem to be isolated (Slatkin's M value far less than 1). However even in MN1, gene flow can be very reduced: populations of South East Kenya (Kitobo, Taita, Mtito) have a very high gene flow with populations of coastal Kenya (Lunga, Kwale) (Slatkin's M value = infinite) (207 km between Kitobo and Lunga) but much less with Northern populations (Mount Kenya 2, Tana, Mwingi) (Slatkin's M value = 1.96) (304 km between Kitobo and Mount Kenya 2), whereas the gene flow is still much more reduced between northern and southern Kenya (Slatkin's M value = 0.84) (486 km between Mount Kenya 2 and Lunga).

Discussion

The three species are morphologically very similar but can however be distinguished by their habitus. To the naked eye *M. nubifera* appears in most cases dark, without visible elements of pattern, whereas in *M. melanodonta* the distal upper end of the forewing, including the reniform, appears like a large rounded pale spot. *M. fuliginosa* is much smaller than the other two species and has no pale spot but is neither so homogeneously coloured nor as dark as *M. nubifera*. Its hind wing is also much greyer than in the other two species, in which this is nearly white.

Some intraspecific variation was detected in the male genitalia (figs. 13 & 14), particularly with regard to the number and shape of spines at the extremity of the sclerotized band of the aedeagus. The number of spines was variable within species (between one and four spines) and did not enable the distinction of the different species. Some differences in shape were observed, but they were not species-characteristic: the spines were curved in *M. melanodonta* but only in populations from Mozambique and Uganda, and straight in the Kenyan population (Race Course) as it was the case of *M. nubifera*. In *M. fuliginosa* the spines were curved like in the first two populations of *M.*
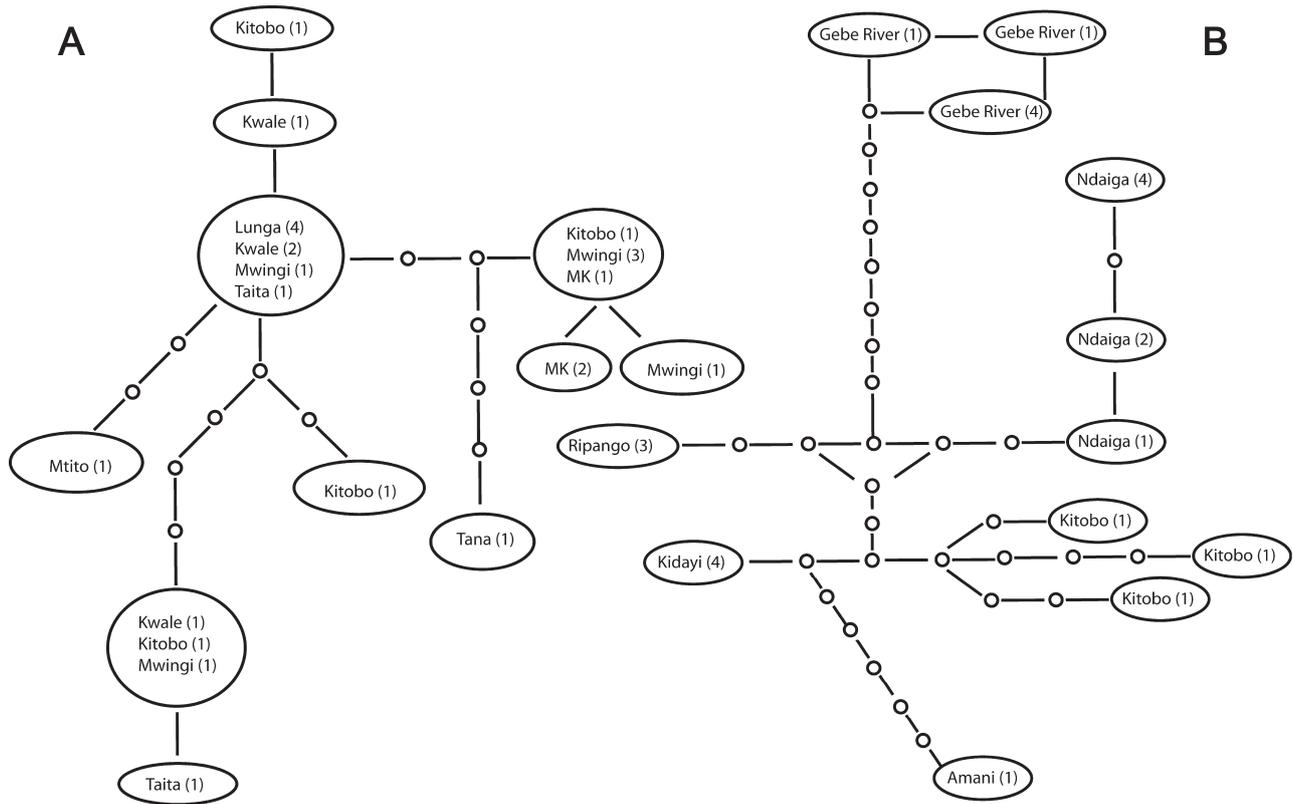
Figure 12
Haplotype networks of the clades of *Manga nubifera* (A. MN1, B. MN2).

melanodonta but sharper. However the small number of specimens examined does not permit to conclude whether this is a characteristic of that species or not.

The shape of the clasper and cucullus showed in *M. melanodonta* some slight differences between various areas, but it is unclear at present if more sampling in intermediate regions would not show a cline. Figure 14 shows for instance that in Uganda the clasper is rather slender and the cucullus clearly triangular, in Kenya the clasper is fairly similar but the cucullus more rounded and in Mozambique the clasper is wider and longer with a shorter cucullus. No such geographic variability was observed within *M. nubifera*.

The observations made during this study allow

Table 3. Infraspecific diversity (value ± SD) in *M. nubifera.*

Clade	MN1	MN2
Gene diversity	0.860±0.050	0.931±0.028
Nucleotide diversity	0.00439±0.00252	0.01025±0.0054
Pairwise differences	4.13±2.13	9.64±4.58

to better define the characteristics and species composition of the genus *Manga*. In fact, the examination of the types of *M. bisignata* Laporte 1973, described from Cameroun (Laporte 1973), showed that *M. bisignata* is wrongly placed here and represents a synonym of *Busseola quadrata* Bowden 1956 (**n. syn.**). Both specimens have the same habitus, and the male genitalia are identical in all respects (tegumen, valve, juxta, vinculum, aedeagus). The genus *Manga* presently includes then five species: *M. basilinea* Bowden 1956 (fig. 15), the type species of the genus, from West Africa, *M. belophora* Fletcher 1961 (fig. 16) from Ruwenzori and the three species collected in this study: *M. melanodonta*, previously known only from Uganda (Hampson 1910), *M. nubifera* known until now only from Congo forest (Hampson 1910), and *M. fuliginosa*, found only in Mozambique.

The molecular analysis is in agreement with the morphological observations. The three morphological species are clearly distinct at the molecular level. The three morphological types observed in the regional populations of *M. melanodonta* belong to three
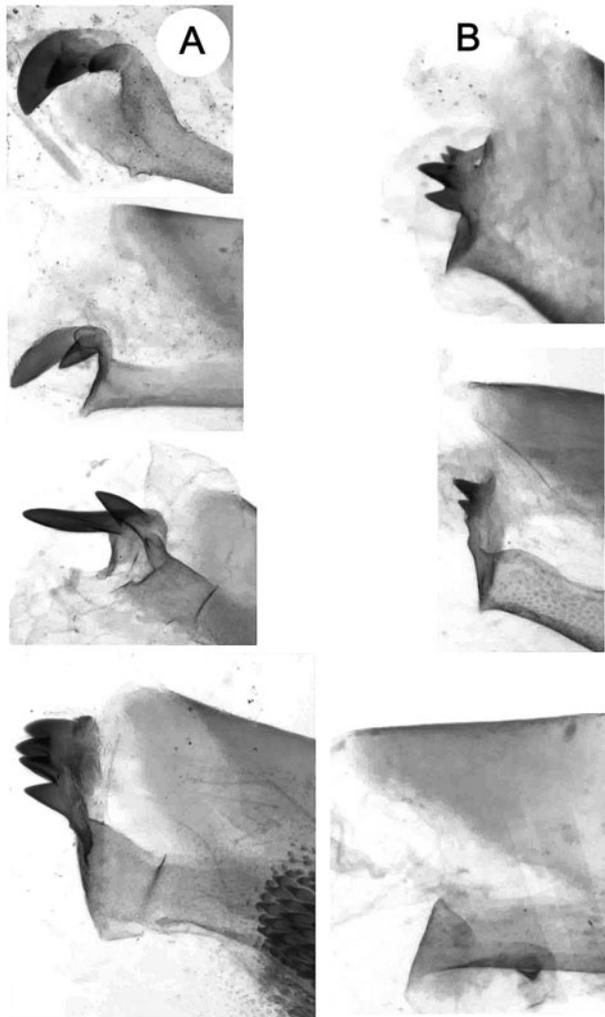
Figure 13
Variability in the shape and the number of spines of the sclerotized band of the aedeagus. A. *Manga melanodonta* (from up, specimens from Uganda, Mozambique, and two specimens from Kenya); B. *Manga nubifera*: three specimens from Kenya.

Figure 14
Variability in clasper and cucullus shape in *Manga melanodonta*. A. 2 specimens from Mozambique; B. 1 specimen from Uganda; C. 2 specimens from Kenya.

different clades, which suggests that they could be considered presently as sub-species, genetically isolated by distance. However the Uganda and Kenya clades are so close geographically that this isolation may vanish in the near future. The molecular data show moreover a similar fragmentation inside *M. nubifera* that was not detected morphologically, although the genetic isolation between both clades was apparently complete until very recently. They indicate also more recent diversification events that were not morphologically visible. With the exception of *M. fuliginosa*, for which only two males were collected from one locality, the molecular study revealed a complex evolutionary history of the group, with successive diversification events, migrations and colonizations of new areas.

The study of the intraspecific diversity of *M. nubifera* shed a light on the process of colonization during the recent past. It showed that the populations of Tanzania (Amani, Kidayi) seemed to have a different history from those of Mozambique (Ripango). The phylogenetic tree indicated that the population from Uganda (Ndaiga) was ancestral: the haplotype network suggests that the colonisation of the different areas occurred recently and that it followed different routes. The first one to Ethiopia (Gebe River) was probably due to a small population that was quickly isolated, favouring rapid genetic drift. A second route, eastern to Lake Victoria, enabled the colonization of Tanzania from which a population invaded South Kenya (Kitobo forest). The Kitobo forest is indeed a Tanzanian type forest, the north-eastern end of the Zambezian miombo ecological region (White 1986). Some excentred populations of Tanzania like Amani have likely small sizes and little gene flows with other populations and have a quicker rate of fixation. The Mozambique (Ripango) population seems to originate from a third route, western to Lake Victoria. It is genetically close to the Uganda population, in spite of a long geographic distance. This suggests that either
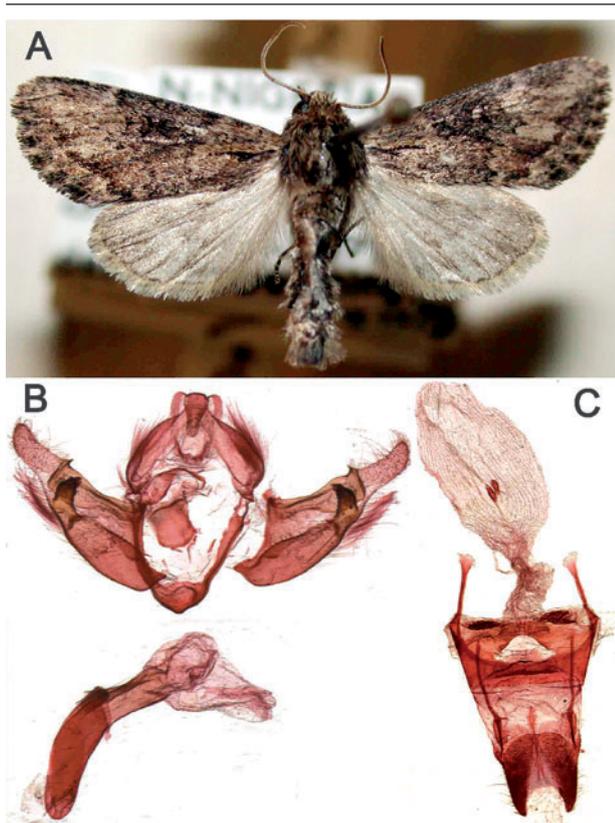
Figure 15
Manga basilinea. A. Male, type adult; B. Male genitalia; C. Female genitalia.
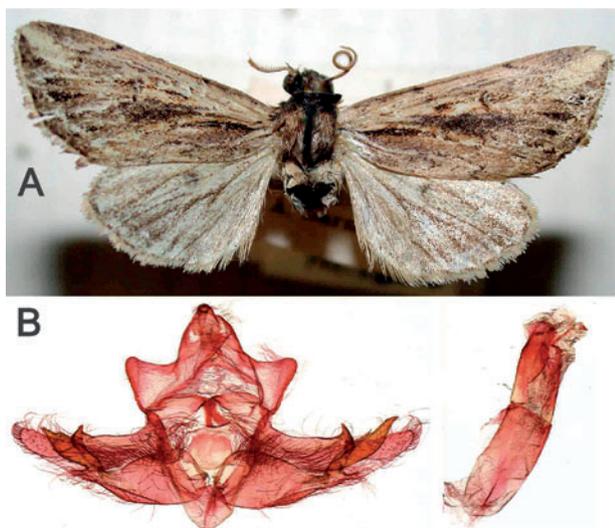
Figure 16
Manga belophora. A. Male, type adult; B. Male genitalia.

the colonization of Mozambique is very recent, or that the size of this population is high, reducing the genetic drift. Local populations were very homogeneous (only one haplotype for instance in Kidayi, or in Ripango, only 3 very close in Gebe River) except in Kenya and Uganda. This study showed also the influence of mountains in the isolation of populations. Indeed, the gene flow estimated by Slatkin's M value indicates that populations of south east and coastal Kenya can be considered as a single population when strong limitations to gene flow occur with the northern population. The probable cause of this isolation is not the geographic distance but the Machakos mountains that constitute a geographical barrier between both groups.

Independently of the morphological similarities, two facts suggest that the evolution of the group is recent. The first one is the impossibility of estimating the divergence dates using the method proposed by Gaunt & Miles (2002), because of the absence of variation of the slowly evolving sites they used. Indeed, the comparison by Gaunt and Miles of the pairwise difference (difference of 21 aminoacids among 477 for the whole cox1 gene) and the estimated date of divergence (about 100 million years) between the two moth species they used, *Manduca sexta* (L. 1764) (Sphingidae) and *Feltia jaculifera* (Guenée 1852) (Noctuidae), shows that only divergence events older than about 5 million years are likely to be dated (since, when considering a local molecular clock, about one aminoacid mutation occurred every 5 million years). For the part of the cox1 we sequenced (298 amino acids) a pairwise difference of 11 aminoacids was observed between both species. The evolutionary rate is then not very different from (and even a little slower) that of the whole gene (3.70% vs 4.40%), and recent divergence events could not be estimated. This result suggests however that the observed divergence events between *Manga* species are recent, and could have occurred in the last 5 million years. The second fact is the high host plant specialization of these insects, and more generally of the African noctuid stem borers, that is now demonstrated (Le Rü *et al.* 2006). Indeed, this specialization occurred on tropical monocot plants with C4 photsynthetic system. And it is known that C4 monocots were very rare before 9 Myr before present (BP) and became dominant and highly diversified between 9 and 4 Myr BP, when they replaced C3 plants (Jacobs *et al.* 1999). The borer specialization on these plants, that probably contributed to their diversification, could then only occur after the host plant diversification. The borer diversification may moreover have occurred rather a long time after that of host plants, resulting in sequential radiation such as observed in other insects *e.g.*

psyllids (Percy *et al.* 2004). The results show that such a case likely occurred for *M. melanodonta*, that colonized *Setaria* species recently, probably long after the plant diversification.

From these two facts it can be concluded that the evolution rate of the studied species is probably close to that estimated by Brower, since the calculated divergence dates were less than 5 million years. Moreover, the comparison of the estimated divergence dates with the main paleo-climatic events that occurred in Africa in the past five million years enables to confirm this rate. Indeed, in addition to the part played in divergence by the host plant specialization, the influence of major paleo-climatic events (dry and cold periods that favoured speciation processes by isolation in refuges followed by humid and warm periods favouring dispersion), that occurred simultaneously in vast regions, is suggested by the similar genetic distance observed between infraspecific clades. The comparison of the estimated divergence dates with the occurrence of these major events shows that both match, which enables to propose the following scenario for the evolution of the group.

1. The ancestor of the group lived in austral Africa in stems of *P. maximum*. A first fragmentation event occurred about 4.6 Myr BP, which resulted in the species *M. fuliginosa* and the ancestor of *M. melanodonta* and *M. nubifera*. An important environmental event occurred at that time in this region (Lovett 1993): the reinforcement of the Benguela current that occurred around 5 Myr BP, resulting in a greater aridity in south western Africa and a compression of the southern Guineo-congolian forests. These conditions were favourable for speciation and then it can be supposed that both events are related.

2. From this time until about 3.5 Myr BP the African climate was humid (Lovett 1993; De Menocal 1995), favouring colonization of new areas. The ancestor of *M. melanodonta-M. nubifera* probably extended towards the northeast. Maybe the gene flow between the northern population, that later resulted in *M. nubifera*, and the southern, which resulted in *M. melanodonta*, reduced progressively. The divergence in environmental preferendums between both populations (dry forests and one host plant for *M. nubifera*, humid forests and several host plants for *M. melanodonta*) may have begun then or during the fragmentation event that occurred next.

3. The fragmentation between both species occurred around 3 Myr BP. This was the first result of several successive cycles of dry-humid periods that occurred between 3.4 and 1.7

Myr BP. This period was very favourable to speciation in other groups such as mammals (Bobe & Behrensmeyer 2004), which showed a high species turn-over. Particular dry and cold periods occurred around 2.5 Myr BP (Bonnefille 1983) and 1.7 Myr BP (De Menocal 1995). For *Manga* species, south-north migrations were no longer possible at this time, resulting in the divergence of both species. The colonization by *M. melanodonta* of new host plants belonging to *Setaria*, if not yet done, may have occurred when the species was limited to altitudinal forested refuges during the dry periods.

4. In spite of these successive cycles no other still visible fragmentation event occurred in *Manga* during about 1 Myr, maybe because of the shortness of the cycles (around 200 thousand years). On the contrary, this period was probably favourable to the colonization of new areas: it can be assumed that *M. nubifera* colonized both sides of the Rift Valley, with some isolation by distance beginning, and that *M. melanodonta* began to migrate northwards. Humid periods favoured apparently much more the expansion of the species adapted to humid environment, thanks particularly to the specialization on *Setaria*.

5. At about 2 Myr BP, a new fragmentation occurred in both species, resulting in the two clades of *M. nubifera*, and the three clades (Mozambique, Kenya, Uganda) of *M. melanodonta*.

6. Around 1 Myr BP, a new strong dry period occurred (De Menocal 1995), which likely resulted in the fragmentation of the Uganda and Kenya clades of *M. melanodonta*. Apparently, it had no influence on *M. nubifera* diversification.

7. From this time, the western clade of *M. nubifera*, as shown by the haplotype network, expanded highly, with the colonization of new areas in many directions: northern to Ethiopia and southern to Tanzania and Mozambique, and the invasion of South Kenya from Tanzania. Most populations of *M. melanodonta* seem to have remained isolated in different regions, but an expansion also occurred in the Kenyan clade west of the Rift Valley, since one individual was found in Taita. This expansion was apparently much more limited than for *M. nubifera*, which would indicate that the conditions of the last million years, with the successive glacial cycles, were not suitable for strong migrations of the species adapted to humid areas, *M. melanodonta*.

In addition to the influence of paleo-climatic events, geological barriers played a part in this evolution. The role of the Rift Valley is thus particularly visible at two levels. First, it explains the isolation of both clades of *M. nubifera* for about 2 Myr (the colonization of the east of the Rift Valley in South Kenya from Tanzania is very recent) and their different expansion. The clade east of the Rift could apparently not expand, when the western clade invaded many new areas. Second, in *M. melanodonta*, the genetic distance between the two clades east and west of the Rift (KT and RC) is higher than the one between the two clades of Uganda. Both fragmentation events are probably due to the same dry period at 1 Myr, but the two Kenyan populations were at that time likely already genetically distant because of previous stronger isolation by the Rift.

Probably of less importance, but favouring local diversification at least temporarily, is the influence of mountains, such as was observed for the Machakos mountains east of the Rift Valley.

From this first study on the evolution of a Noctuid stem borer genus, it can be concluded that the combination of three forces has shaped the diversification of species in the past million years. Most divergence events resulted from paleo-climatic changes, but geological barriers and adaptation to new host plants played also significant parts by themselves or by enhancing the climate effects.

References

Bobe R., Behrensmeyer A.K. 2004. The expansion of grassland ecosystems in Africa in relation to mammalian evolution and the origin of the genus *Homo*. *Palaeogeography, Palaeoclimatology, Palaeoecology* **207**: 399-420.

Bonnefille R. 1983. Evidence for a cooler and drier climate in the ethiopian uplands towards 2.5 Myr ago. *Nature* **303**: 487-491.

Bowden J. 1956. New species of African stem-boring Agrotidae (Lepidoptera). *Bulletin of Entomological Research* **47(3)**: 415-428.

Bowden J. 1976. Stem borer ecology and strategy for control. *Annals of Applied Biology* **84**: 107-134.

Brower A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA* **91**: 6491-6495.

Clement M., Posada D., Crandall K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1659.

Corpet F. 1988. Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Research* **16(22)**: 10881-10890.

De Groote H. 2002. Maize yield losses from stemborers in Kenya. *Insect Science and its Application* **22(2)**: 89-96.

De Menocal P.B. 1995. Plio-pleistocene African climate. *Science* **270**: 53-59.

Fletcher D.S. 1961. *Ruwenzori expedition 1952. Noctuidae*. London, British Museum (natural History). William Clowes and sons Ltd. **1(7)**: 177-323.

Gascuel O. 1997. BIONJ: An improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution* **14(7)**: 685-695.

Gaunt, M.G., Miles M.A. 2002. An insect molecular clock dates the origin of the insects and accords with paleontological and biogeographical landmarks. *Molecular Biology and Evolution* **19(5)**: 748-761.

Guindon, S., Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood. *Systematic Biology* **52(5)**: 696-704.

Hampson, G.F. 1910. *Catalogue of the Noctuidae in the collection of the British Museum*. Taylor and Francis, London, 552 p.

Harris K.M. 1962. Lepidopterous stem borers of cereals in Nigeria. *Bulletin of Entomological Research* **53(1)**: 139-172.

Harry M., Solignac M., Lachaise D. 1998. Molecular evidence for parallel evolution of adaptative syndromes in fig-breeding *Lissocephala* (Drosophilidae). *Molecular Phylogenetics and Evolution* **9**: 542-551.

Hasegawa M., Kishino H., Yano T. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**: 160-174.

Holloway J.D. 1998. Noctuidae, p. 79-86 *in*: **Polaszek A. (ed.),** *African cereal stem borers*. CABI, London.

Jacobs B.F., Kingston J.D., Jacobs L.L. 1999. The origin of grass-dominated ecosystems. *Annals of the Missouri Botanical Garden* **86**: 590-643.

Khan Z.R., Pickett J.A., Wadhams L., Muyekho F. 2001. Habitat management strategies for the control of cereal stemborers and striga in maize in Kenya. *Insect Science and its Application* **21(4)**: 375-380.

Kumar S., Tamura K, Nei M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* **5**: 150-163.

Laporte B. 1973. Descriptions de nouvelles espèces africaines de noctuelles (Insecta Lepidoptera). *Annales de la Faculté des Sciences du Cameroun* **14**: 109-126.

Le Rü B.P., Ong'amo G.O., Moyal P., Muchugu E., Ngala L., Musyoka B., Abdullah Z., Kauma Matama T., Lada V.Y., Pallangyo B., Omwega C.O., Schulthess F., Calatayud P.A., Silvain J.F. 2006. Geographical distribution and host plant ranges of East African noctuid stem borers. *Annales de la Société Entomologique de France (N.S.)* **42(3-4)**: 353-361.

Lovett J.C. 1993. Climatic history and forest distribution in eastern Africa, p. 23-29 *in*: **Lovett J. C., Wasser S. K. (eds)**, *Biogeography and ecology of the rain forests of eastern Africa*. Cambridge University Press.

Monteiro A., Pierce N.E. 2001. Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from COI, COII, and EF-1alpha gene sequences. *Molecular Phylogenetics and Evolution* **18(2)**: 264-281.

Moyal P. 1998. Les foreurs du maïs en Côte d'Ivoire: vers une protection intégrée. *Agriculture et Développement* **19**: 16-27.

Moyal P. 2006. History of the systematics of the *Sesamia sensu lato* group of African noctuid stem borers of monocotyledonous plants (Lepidoptera). *Annales de la Société Entomologique de France (N.S.)* **42(3-4)**: 285-291.

Moyal P., El-Hefnawy N., Fediere G., El-Metwally M.F., Giannotti J., Abol-Ela S. 2002. Biocontrol of the maize borer *Sesamia cretica* Led. (Lepidoptera: Noctuidae) in Egypt using a granulovirus. *International Journal of Pest management* **48(1)**: 49-57.

Onyango F.O., Ochieng'Odero J.P.R. 1994. Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis et Applicata* **73**: 139-144.

Page R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357-358.

Percy D.M., Page R.D.M., Cronk Q.C. 2004. Plant-insect interactions: double-dating associated insect and plant lineages reveals asynchronous radiations. *Systematic Biology* **53(1)**: 120-127.

Polaszek A., Khan Z.R. 2000. Les plantes hôtes, p. 3-10 *in*: **Polaszek A., Delvare G. (eds.)**, *Les foreurs des tiges des céréales en Afrique*. CIRAD-CTA, Montpellier.

Poole R.W. 1989. Noctuidae. Part 2, p. 501-1013 *in*: **Heppner J. B. (ed.)**, *Lepidopterum Catalogus (New Series) Fascicle 118*. E.J. Brill, Flora & Fauna Publications, New York.

Posada D., Buckley T.R. 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**: 793-808.

Posada D., Crandall K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14 (9)**: 817-818.

Sanger F., Nicklen S., Coulson A.R. 1977. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences* **74 (12)**: 5413-5467.

Schneider S., Roessli D., Excoffier L. 2000. *Arlequin ver. 2.000: a software for population genetics data analysis*. Genetics and biometry laboratory, University of Geneva, Switzerland.

Schulthess, F., Bosque-Perez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Goergen G. 1997. Exchange of natural enemies of lepidopteran cereal stemborers between African regions. *Insect Science and its Application* **17(1)**: 97-108.

Simmons R.B., Weller S.J. 2001. Utility and evolution of Cytochrome *b* in insects. *Molecular Phylogenetics and Evolution* **20 (2)**: 196-210.

Simon C., Frati F., Beckenbach A., Crespi B., Liu H., Flook P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**: 651-701.

Slatkin M. 1991. Inbreeding coefficients and coalescence times. *Genetic research* **58**: 167-175.

Swofford D.L. 2003. *PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0*. Sinauer, Sunderland, Massachussetts.

Tajima F. 1993. Simple methods for testing the molecular evolutionary clock hypothesis. *Genetics* **135**: 599-607.

Tams W.H.T., Bowden J. 1953. A revision of the African species of *Sesamia* Guenée and related genera (Agrotidae-Lepidoptera). *Bulletin of Entomological Research* **43**: 645-679.

Templeton A.R., Crandall K.A., Sing C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619-633.

Van Den Berg J., Rebe M., De Bruyn J., Van Hamburg H. 2001. Developing habitat management systems for graminaceous stemborers in South Africa. *Insect Science and its Application* **21(4)**: 381-388.

White F. 1986. *La végétation de l'Afrique*. ORSTOM-UNESCO, Paris, 384 p.

Zakharov E.V., Caterino M.S., Sperling F.A.H. 2004. Molecular phylogeny, historical biogeography, and divergence time estimates for swallowtail butterflies of the genus *Papilio* (Lepidoptera: Papilionidae). *Systematic Biology* **53(2)**: 193-215.

Mitochondrial DNA phylogeography of the *Cotesia flavipes* complex of parasitic wasps (Hymenoptera: Braconidae)

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Abstract. The *Cotesia flavipes* species complex of parasitic wasps are economically important worldwide for the biological control of lepidopteran stem borers. The complex currently comprises three species: *Cotesia flavipes* Cameron, *C. sesamiae* (Cameron) and *C. chilonis* (Matsumura) (Hymenoptera: Braconidae), which appear morphologically similar. Despite their economic importance, little is known about the genetic diversity and phylogeography of these parasitoids. Differences in the biology of geographic populations have generally been interpreted as genetic divergence among strains, but direct genetic evidence is lacking. In Australia, several stem borer pests in neighbouring countries have been identified as significant threats to the sugar industry. However, the status of *C. flavipes* in Australia is unknown. To examine the genetic variation among worldwide populations of the *C. flavipes* complex and investigate the status of the Australian *C. flavipes*-like species, partial sequence data were generated for mitochondrial gene regions, *16S rRNA* and *COI*. Parsimony, minimum evolution and Bayesian analyses based on 21 geographic populations of the complex and four outgroups supported the monophyly of the complex and the existence of genetically divergent populations of *C. flavipes* and *C. sesamiae*. The geographically isolated Australian haplotypes formed a distinct lineage within the complex and were ~3.0% divergent from the other species. The results indicated that historical biogeographic barriers and recent biological control introductions play an important role in structuring lineages within these species. This study provides a phylogeographical context for examining adaptive evolution and host range within biologically divergent strains of the *C. flavipes* complex.

Résumé. Phylogéographie de l'ADN mitochondrial du complexe parasitaire, *Cotesia flavipes* (Hymenoptera : Braconidae). Le complexe d'espèces parasitaires, *Cotesia flavipes*, est économiquement très important pour le contrôle des lépidoptères foreurs de graminées à travers le monde. Ce complexe comprend les espèces suivantes : *Cotesia flavipes* Cameron, *C. sesamiae* (Cameron) et *C. chilonis* (Matsumura) (Hymenoptera : Braconidae), qui apparaissent très semblables morphologiquement. A part leur importance économique, peu d'information est disponible sur leur diversité génétique et leur phylogéographie. Des différences dans la biologie de populations géographiquement isolées ont été interprétées comme une divergence génétique entre races, sans pour autant pouvoir montrer une divergence génétique. En Australie, plusieurs espèces de foreurs considérés comme des ravageurs dans les pays voisins ont été identifiés comme de réelles menaces pour l'industrie sucrière du pays. Cependant, le statut de *C. flavipes* en Australie est inconnu. Afin d'examiner les variations génétiques des populations de *C. flavipes* à travers le monde et d'étudier le statut des espèces semblables à *C. flavipes* d'Australie, l'analyse des séquences de certaines régions de gènes mitochondriaux, ARNr 16s et COI, a été entreprise. Une étude de parsimonie et une analyse d'évolution minimum et Bayésienne menées sur 21 populations géographiquement différentes du complexe d'espèces et 4 extra groupes soutien la monophylie du complexe et l'existence de divergence génétique entre *C. flavipes* et *C. sesamiae*. Les haplotypes australiens géographiquement isolés ont formé une lignée distincte au sein du complexe d'espèces et ont divergés de 3,0 % du reste des espèces. Les résultats indiquent que des barrières biogéographiques anciennes et des introductions récentes d'espèces de parasitoïdes pour le contrôle biologique ont joué un rôle important dans cette structuration en lignées au sein des espèces. Cette étude permet d'examiner dans un contexte phylogéographique l'évolution de l'adaptation à l'environnement et à l'hôte de races biologiquement divergentes du complexe de parasitoïdes *C. flavipes*.

Keywords: *Cotesia flavipes* complex, phylogeography, stem borer, biological control, Africa.

The species of the *Cotesia flavipes* species complex are gregarious endoparasitoids of lepidopteran stem borers and have been successfully implemented in biological control programs worldwide against these pests. The complex comprises three nominal species: *Cotesia flavipes* Cameron 1891, *C. sesamiae* (Cameron 1906) and *C. chilonis* (Matsumura 1912) that appear morphologically similar. The species within the complex are considered endemic to the following areas: *C. flavipes* to the Indo-Australasia region; *C. sesamiae* to central and southern Africa; *C. chilonis* to eastern Asia, including Japan (Polaszek & Walker 1991; Kimani-Njogu & Overholt 1997). However, worldwide importation of these species has resulted in a more widespread distribution (Polaszek & Walker 1991). *Cotesia flavipes* in particular, has been imported into over 40 countries since 1950 for introductions against stem borer species, such as *Chilo partellus* (Swinhoe 1885), *Diatraea saccharalis* (Fabricius 1791) and *Chilo sacchariphagus* (Bojer 1856) (Polaszek & Walker 1991; Overholt 1998). It now occurs in the Caribbean, major parts of North and South America, east Africa, the Indian Ocean islands, Madagascar, Mauritius and Réunion, and has also been redistributed within Asia (Mohyuddin *et al.* 1981).

Despite their economic importance as biological control agents, very little is known about the genetic diversity and phylogeography of these wasps. The genetic structure of the complex is likely to be influenced by biogeographical pattern and host-parasitoid coevolutionary history. Several authors have recorded variation in the ecology and host-searching behaviour of geographic populations of the *C. flavipes* complex, suggesting the existence of host and/or plant specific strains (Mohyuddin 1971; Mohyuddin *et al.* 1981; Inayatullah 1983; Polaszek & Walker 1991; Ngi-Song *et al.* 1995; Ngi-Song *et al.* 1998; Mochiah *et al.* 2001). However, Potting *et al.* (1997a), demonstrated that *C. flavipes* lacked the ability of adult early learning in different host/plant development and emergence environments. Thus, indicating that differences among *C. flavipes* populations were not due to host/plant selection behaviour among strains, but rather symptomatic of geographic variation in host-parasitoid physiological compatibility and reproductive success (Wiedenmann & Smith 1995; Potting *et al.* 1997b). The interspecific taxonomic history of the complex is somewhat confusing due to difficulty in distinguishing the three species using external morphology (Wilkinson 1928; Watanabe 1932; 1965; Alam *et al.* 1972; Ingram 1983; Polaszek & Walker 1991). Polaszek & Walker (1991) identified the male genitalia as the only reliable morphological character

for separating the species within the complex into two morphospecies: *C. flavipes* and the *C. sesamiae/C. chilonis* subcomplex. Whereas, Kimani-Njogu *et al.* (1997) recognised three somewhat overlapping species based on principal component analysis of 16 morphometric characters. More recently, the status of *C. flavipes* complex has been investigated using allozyme electrophoresis (Kimani-Njogu *et al.* 1998) and DNA sequence data (Smith & Kambhampati 1999; Michel-Salzat & Whitfield 2004). Variation in allozymes at 14 loci provided evidence for genetic variation among populations in the complex, however, there was some suggestion that geographic populations of *C. flavipes* were polyphyletic (Kimani-Njogu *et al.* 1998). Nucleotide sequence data have demonstrated the monophyly of the complex, yet the relationships within the complex change depending on the genes studied (Smith & Kambhampati 1999; Michel-Salzat & Whitfield 2004).

The genetic diversity of the *C. flavipes* complex, therefore, is an area that requires more research. Although members of the *C. flavipes* complex are recorded from numerous stem borer species, certain populations have a more restricted host range (Rajabalee & Govendasamy 1988; Potting *et al.* 1997b). This is best illustrated in Australia where major stem borer pest species are not present (Allsopp *et al.* 2000) and there is only one recorded host for the species recognised as *C. flavipes* (Sallam & Allsopp 2002). Although *C. flavipes* is considered endemic to the Indo-Australian region, its status within Australia is unknown. Based on external morphology, the native parasitoid species, *Apanteles nonagriae* Olliff 1893, was synonymised with *C. flavipes* (Austin & Dangerfield 1992). Records of *A. nonagriae* in Australia extend back to the 1920s when it was first recorded parasitising the native stem borer *Phragmatiphila truncata* (Walker 1856) (now *Bathytricha truncata*) in South Mulgrave, Australia in sugarcane (Jarvis 1927). This report also indicated that the parasitoid had been recorded previously parasitising 50% of *B. truncata* larvae infesting rice in New South Wales. The use of *C. flavipes* for biological control is of particular interest to the Australian sugar industry as a number of stem borer pests have been identified as significant threats to quarantine (FitzGibbon *et al.* 1999). These pest species belonging to the genera *Chilo* Zincken 1817, *Sesamia* Guenée 1852, *Scirpophaga* Treitschke 1832 are widely distributed throughout south-east Asia, Indonesia and Papua New Guinea and cause considerable damage and production losses in sugarcane and other cereal crops (Kalshoven 1981; Young & Kuniata 1992; Lloyd & Kuniata 2000). For instance, in Papua New Guinea, infestation by *Sesamia*

grisescens Warren 1911 resulted in losses up to US $8.4 million and a decreased sugar yield of 5-18% in the early 1990s (Kuniata & Sweet 1994).

An understanding of the genetic variation within geographic populations of the *C. flavipes* complex can provide a phylogeographical context for examining host range. Geographic isolation and restricted host use of the Australian *C. flavipes* is likely to lead to genetic divergence from other populations. This can have important implications for the use of these parasitoids

in biological control programs against invading exotic pest species. A major obstacle that impedes biological control implementation is insufficient taxonomic information of both natural enemies and target pests (Danks 1988; Schauff & LaSalle 1998). Adequate systematics and accurate identification can prevent delays, wasted resources and failure of programs (Schauff & LaSalle 1998). Therefore, it is necessary to develop clear diagnostic characters prior to making biological control releases within Australia. The aims

Table 1. The identity, collection locations, host/habitat information, number of individuals (*n*), source and GenBank accession numbers for taxa analysed in this study. Source = lab colony or field collection.

Species	Locality	Host	Habitat	n /source	16S rRNA	COI
Cotesia flavipes	Thailand	Chilo tumidicostalis	Sugarcane	3/field	DQ232357	DQ232340
	Piracicaba, Brazil	Diatraea saccharalis	Sugarcane	2/colony	DQ232348	DQ232320
	India	Chilo partellus	Maize	5/ field	DQ232352	DQ232336
	Florida, USA	Diatraea saccharalis	Sugarcane	2/ colony	DQ232350	DQ232330
	Mombasa, Kenya	Chilo partellus	Maize	2/field	DQ232351	DQ232317
	Mandeville, Jamaica	Diatraea saccharalis	Sugarcane	1/field	DQ232349	DQ232340
	south Pakistan	Chilo partellus	Maize	2/colony	DQ232353	DQ232335
	Sri Lanka	Chilo sacchariphagus	Sugarcane	1/field	DQ232354	DQ232327
	Labour-donnais, Mauritius	Chilo sacchariphagus	Sugarcane	1/field	DQ232347	DQ232319
	south Sumatra, Indonesia	Sesamia inferens	Sugarcane	2/field	DQ232356	DQ232337
	Ramu, PNG	Sesamia grisecens	Sugarcane	3/field	DQ232346	DQ232316
	Réunion	Chilo sacchariphagus	Sugarcane	1/field	DQ232355	DQ232329
	Okinawa Prefecture, Japan	Sesamia inferens	Sugarcane	2/field	DQ232361	DQ232328
Cotesia sp.	Giru, Australia	Bathytricha truncata	Sugarcane	5/field	DQ232358	DQ232322
Cotesia sp.	Bundaberg, Australia	Bathytricha truncata	Sugarcane	5/field	DQ232360	DQ232323
Cotesia sp.	Mackay, Australia	Bathytricha truncata	Sugarcane	5/field	DQ232359	DQ232333
Cotesia chilonis	Illinois, USA (originated from Japan)	D. saccharalis (Chilo supressalis)	Sugarcane (rice)	1/colony	DQ232345	DQ232339
Cotesia sesamiae	Mombasa, Kenya (Hap.1)	Sesamia calamistis	Maize	1/field	DQ232344	DQ232325
	Mombasa, Kenya (Hap. 2)	Chilo partellus	Sorghum	1/field	DQ232341	DQ232318
	Kitale, Kenya	Busseola fusca	Sorghum	1/field	DQ232342	DQ232324
	Pietersburg, South Africa	Chilo partellus	Maize	1/field	DQ232343	DQ232326
Cotesia glomerata	University of Adelaide, Australia				DQ232364	DQ232331
Cotesia rubecula	University of Adelaide, Australia				DQ232365	DQ232332
Cotesia marginiventris	University of Adelaide, Australia				DQ232363	DQ232338
Cotesia urabae	Hobart, Tasmania, Australia		Eucalyptus sp.	1/ field	DQ232362	DQ232334

of the present study were to 1) characterise genetic differences among geographic populations of the *C. flavipes* complex on a worldwide basis using mtDNA markers, 2) validate the status of the Australian native *C. flavipes*-like species and, 3) develop a solid phylogeographic framework for the *C. flavipes* complex as a prelude to any application to import specific 'strains' for biological control purposes.

Materials and Methods

Taxonomic sampling, mtDNA extraction and sequencing

The specimens used in this study were collected from three sugarcane-growing localities in Queensland, Australia, or sourced internationally with host/habitat information. Parasitoids were either reared from wild collected hosts or sampled from colonies in research laboratories. The origin and host/habitat information of the 21 populations of the *C. flavipes* complex and four outgroups is provided in table 1 along with the GenBank accession numbers for the sequence data. All outgroups except for *Cotesia urabae* Austin and Allen 1989 were source from The University of Adelaide Insect Collection.

All specimens were stored in 100% ethanol and preserved at -20 °C until DNA was extracted. Total genomic DNA was extracted from headless, whole wasps using the Gentra Systems Puregene® DNA Purification Kit (GentraSystems 2005). Regions of the mitochondrial *cytochrome c oxidase subunit I (COI)* and *16S ribosomal RNA* genes were used for phylogenetic analysis. Universal primers C1-J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTCC-3') and C1-N-2329 (5'-ACTGTAAATATATGATGAGCTCA-3') (Simon *et al.* 1994) were used to amplify a partial fragment of the *COI* gene. Whereas, a partial fragment of *16S rRNA* was amplified using 16SWb (5'-CACCTGTTTATCAAAAACAT-3') (Dowton & Austin 1994) and 16S outer (5'-CTTATTCAACATCGAGGTC-3') (Whitfield 1997).

Polymerase chain reaction (PCR) amplification was carried out in an Eppendorf thermal sequencer. Each 25 µl reaction comprised PCR buffer, 0.2 mM of each dNTP, 0.5 µM of each forward and reverse primers, 2mM MgCl₂, 0.5 units of AmpliTaq Gold® DNA Polymerase (Applied Biosystems Inc.) and 25-100 ng of genomic DNA. PCR conditions were: denaturation at 95 °C for 5min, followed by 35 cycles of 95 °C for 45 sec, annealing at 50 °C for 45 sec, and extension at 72 °C for 30 sec. Final extension was at 72 °C for 3 min. PCR products were purified using the Ultraclean™ PCR Clean-up™ Kit (MoBio Laboratories inc.) and sequenced using ABI Big Dye Terminator Chemistry (Applied Biosystems, Foster City, CA, USA) in 20 µl reaction volumes. Fragments were resolved

on an ABI 3700 capillary sequencer (Applied Biosystems).

Sequence alignment and phylogenetic analysis

Sequences were initially checked in EditView 1.0.1 ABI automated DNA sequence viewer (Applied Biosystems) and resequenced with the reverse primer if ambiguous. Sequences were edited and aligned manually using BioEdit Sequence Alignment Editor version 7.0.1. (Hall 1999). Regions of the *16S rRNA* alignment were highly conserved, thus gaps were easily inferred by eye and confirmed using ClustalX v.1.83 (Chenna *et al.* 2003). Protein-encoding *COI* nucleotide sequences were translated into amino acid sequences using the toggle translation option. The presence of nucleotide saturation in the *COI* 3rd codon position was examined by plotting observed transitions and transversions against genetic divergence.

Phylogenetic reconstructions were conducted for the two mtDNA genes both separately and combined using maximum parsimony (MP), minimum evolution (ME) and Bayesian criteria of optimality. MP/ME analyses, base frequency and pairwise distance calculations were performed using PAUP* version 4.0b10 (Swofford 2000). MP analyses were conducted using equal weights for all positions, with gaps treated as missing data. The model of substitution for ME analyses and for the corrected pairwise distance calculations was selected with Hierarchical Likelihood Tests (hLRTs) performed in Modeltest version 3.6 (Posada & Crandall 1998). For MP and ME methods the heuristic search algorithm options were used with stepwise addition and 100 random taxon addition sequence replicates. Support values for each node (BSV) were examined by bootstrap analyses (Felsenstein 1985) from 1000 pseudoreplicated data sets searched with full heuristic searches.

Bayesian analysis was implemented using MrBayes version 3.0b4 (Huelsenbeck & Ronquist 2001), incorporating the model chosen by Modeltest. The model parameters were unlinked and estimated separately for the *16S rRNA* and *COI* partitions. Analyses were run for two million generations sampling the four Markov chains every 100 generations. Stationarity was determined from plotting log likelihood scores of the sampled trees against generation time. Trees with likelihood scores lower than those at stationarity were discarded as burn-in. Bayesian posterior probabilities of nodes (P_{bay}) were estimated based on the 50% majority rule consensus of the trees. Multiple runs were performed to assess that all parameters were not considerably different at stationarity based on alternate prior probabilities.

Results

A total of 922 base pairs (bp) were sequenced for phylogenetic analysis (*COI* = 552 bp, *16S rRNA* = 370 bp) of which 146 characters were variable and 82 were

Table 2. The corrected (F81+ G) pairwise distance calculations within and among clades of the *C.flavipes* complex and outgroups.

	Outgroups	Clade I	Clade II	Clade III	Clade IV	Clade V
Outgroups	0.071-0.145					
Clade I	0.118-0.188	0.0-0.011				
Clade II	0.122-0.176	0.009-0.019	0.0-0.003			
Clade III	0.114-0.154	0.030-0.034	0.028-0.034	0		
Clade IV	0.106-0.150	0.030-0.039	0.028-0.037	0.017-0.020	0.0-0.002	
Clade V	0.073-0.159	0.042-0.086	0.042-0.076	0.039-0.057	0.030-0.057	0.0-0.028

parsimony informative. Sequences determined here have been deposited in GenBank under Accession Nos. DQ232316-DQ232365. There were no significant differences found in base frequencies across taxa (χ^2 = 5.2 (df = 72), P = 1.00) and base frequency data showed a 79.7% bias in A-T sequence composition (47.2% A, 10.6% C, 9.7% G, 32.5% T) consistent with other apocritan Hymenoptera (Dowton & Austin 1995; Danforth *et al.* 1998; Whitfield & Cameron 1998). Haplotype diversity within collection locations was determined if more than one individual from a locality was sequenced. Only one haplotype was found per collection locality except in Australia where 15 individuals yielded three haplotypes with each haplotype being restricted to a single location. Phylogenetic reconstructions based on the two mtDNA regions and the combined data from the genes yielded congruent trees with nearly identical topologies. Therefore, for the purpose of this paper, the data have

been combined. The optimal model of evolution for the dataset estimated by Modeltest was the F81+G model (Felsenstein 1981). The parameters specified under this model were: base frequencies of A = 0.45, C = 0.11, G = 0.08, T = 0.42, and a shape parameter for the gamma distribution (α = 0.078).

MP analysis yielded 10 equally parsimonious trees, each with a tree length of 219 steps, consistency index (CI) = 0.735 and retention index (RI) = 0.825, while ME analysis yielded a total of 39 trees (ME score = 0.21868) (fig. 1). MP, ME and Bayesian analyses produced trees with similar topologies showing five major ingroup clades (I-V) (figs 1 & 2). The corrected pairwise distance calculations within and among clades are listed in Table 2. Saturation plots of transversions and transitions in the *COI* 3rd codon position showed no evidence of saturation within the ingroups and all analyses exhibited strong support for the monophyly of the *C. flavipes* complex.

Cotesia sesamiae and *C. chilonis* (Clade V) showed a
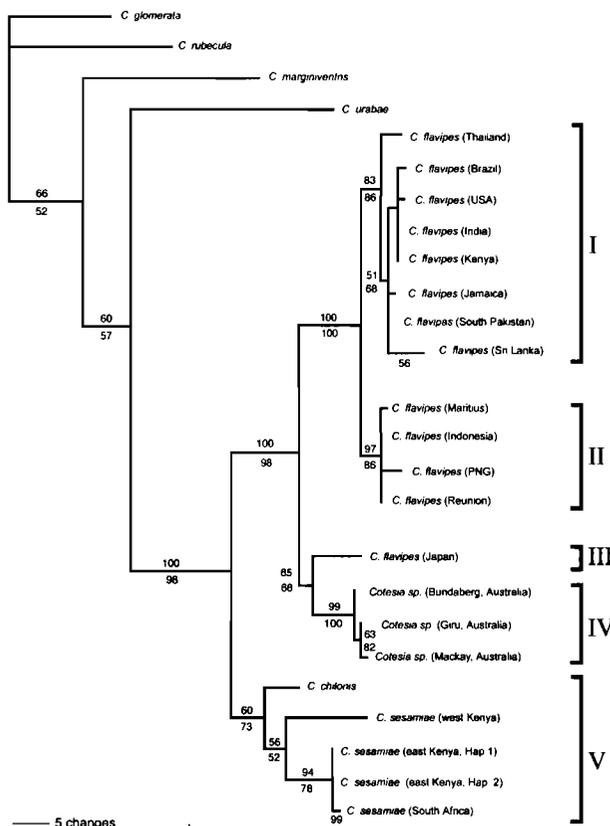
Figure 1
One of the equally most parsimonious trees derived from partial *16S rRNA* and *COI* mtDNA nucleotide sequence data from geographic populations of the *Cotesia flavipes* complex (Clades I-V) and four outgroups. The numbers indicate bootstrap proportions ≥ 50% values from 1000 pseudoreplicates of the MP analysis (above the nodes) and ME (below the nodes).
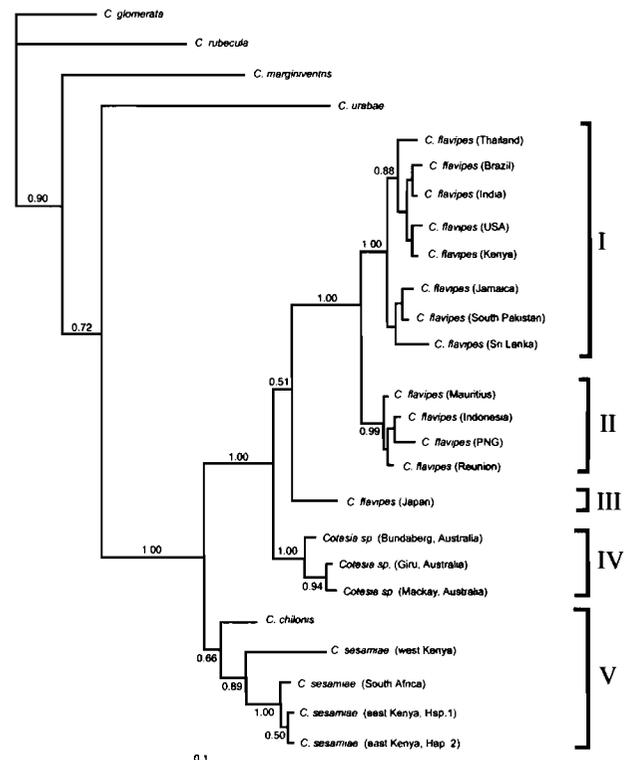
Figure 2
Bayesian tree derived from partial *16S rRNA* and *COI* mtDNA nucleotide sequence data from geographic populations of the *Cotesia flavipes* complex (Clades I-V) and four outgroups. The numbers represent Bayesian posterior probabilities ≥ 50%.

pairwise sequence divergence of 3.9-8.6% with respect to *C. flavipes* and the Australian samples. There was generally low bootstrap support (BSV < 73%, P_{bay} = 66) for the relationship between *C. chilonis* and *C. sesamiae* and these two taxa were divergent by 2.0 –2.8%. There is some evidence for the existence of strains within the *C. sesamiae* group (Clade V) with two haplotypes from east and west Kenya found on different hosts. Interestingly, the Australian populations were genetically divergent from other populations of *C. flavipes* and formed the sister group to clades I-III in the Bayesian analysis (P_{bay} = 1.00) (fig. 2) and the sister group to clade III alone from Japan in the MP/ME analyses (BSV < 70 %) (fig. 1). Within the *C. flavipes* sensu stricto there is support for three clades (I, II & III), and these lineages with the Australian clade (IV) form a monophyletic group.

Discussion

The purpose of this study was to examine phylogeographic divergence within the *Cotesia flavipes* complex and compare the Australian *Cotesia* species with worldwide populations of *C. flavipes*. The results indicate that geography plays an important role in structuring lineages within these species. Phylogenetic reconstruction of the *C. flavipes* complex supported the monophyly of the complex, as have others studies using allozyme and nucleotide sequence data (Kimani-Njogu *et al.* 1998; Smith & Kambhampati 1999; Michel-Salzat & Whitfield 2004). The mtDNA sequence divergence among the members of the complex corresponds to historical biogeographic barriers of these three species; *C. flavipes* from the Indo-Australasian region, *C. sesamiae* from Africa and *C. chilonis* from East Asia and Japan. However, the relationships within the complex are only partially resolved using *16S rRNA* and *COI* sequence data. The reconstructions showed *C. sesamiae* and *C. chilonis* as sister species, however support for this relationship was low. In contrast, Michel-Salzat & Whitfield (2004) distinguished a separation of these two species with phylogenetic analyses of *16S rRNA, NADH1, 28S rRNA and LW opsin* genes, showing *C. sesamiae* as most basal in the complex and *C. chilonis* and *C. flavipes* as sister taxa. In the past, there has been some debate over the validity of their species status. As mentioned above, no morphological characters have been established to distinguish the two species, whereas *C. flavipes* can be separated based on male genitalic morphology (Polaszek & Walker 1991). Likewise, Kimani & Overholt (1995) conducted interspecific crosses of the three species and found a unidirectional cross of *C. sesamiae* males and *C. chilonis* females resulted in viable female progeny, implying that gene flow could occur if these species were sympatric. The present study showed 2.0-2.8% sequence divergence between *C. sesamiae* and *C. chilonis*, however only one specimen of *C. chilonis* was available for analysis. Given the divergence found within intraspecific populations of the complex we are unable to confirm that the divergence found between this taxon and populations of *C. sesemiae* is relevant regarding the species status of *C. chilonis*.

The phylogeography of intraspecific populations of the complex corresponds to biogeographic barriers to dispersal and the movement history of these species for biological control introductions. This study adds a new dimension to past research carried out on the *C. flavipes* complex and expands on research by Kimani-Njogu *et al.* (1998) that provided genetic evidence for variation among eight populations in the complex using allozyme data. In the present study the mtDNA data from 21 populations of the complex indicated genetic divergence in geographic populations of *C. flavipes* and *C. sesamiae*. Four distinct clades were found within the whole *C. flavipes* group; haplotypes from Thailand, Brazil, India, Kenya, USA, Jamaica, South Pakistan and Sri Lanka formed Clade I; haplotypes from Mauritius, Réunion, Papua New Guinea (PNG) and Indonesia formed Clade II; the Japan haplotype formed Clade III and; the Australian haplotypes formed Clade IV (figs 1 & 2).

The geographically isolated Australian and Japanese haplotypes formed distinct lineages within the complex and were ~3.0% divergent from the other *C. flavipes* haplotypes. The divergence of the Australia taxon is an interesting finding for the Australian sugar industry. Although, the data do not provide conclusive evidence for the existence of a discrete species, it does raise questions on its reproductive isolation from other populations and the ability of this taxon to effectively parasitise stem borer pests that pose a threat to the industry. Lower levels of sequence divergence between reproductively isolated strains have been found in other Hymenoptera species. For instance, Hufbauer *et al.* (2004) found divergence of 1.6% using a larger fragment from *COI* and *COII* between partially reproductively isolated populations of the braconid wasp, *Aphidius ervi* Haliday 1834. Likewise, Heimpel *et al.* (1997) found sequence variation of 2% in a larger fragment of *16S rRNA* and reproductive isolation between two strains of the braconid *Bracon hebetor* Say 1836. Preliminary findings on the life history traits of these populations suggest there may be differences in biology when compared to *C. flavipes* and *C. sesemiae* (Muirhead, *in. lit.*). Thus, it appears that the Australian taxon, formally described as *Apanteles nonagriae* and

synonymised with *C. flavipes*, may be a cryptic *Cotesia* species that is closely related to *C. flavipes*. However, further studies are needed to examine the male genitalic morphology and to investigate the ability of these haplotypes to mate with other species in the complex. This research combined with the addition of nuclear markers will help to delineate species boundaries. Moreover, experimental data are needed to determine the reproductive success of Australian *Cotesia* on high threat pests to the Australian sugar industry.

The movements of species within the complex are not easily traced, as many reports of attempted introductions remained unpublished (Polaszek & Walker 1991). However, the sources of several *C. flavipes* introductions are recorded from India and Pakistan. During the 1970's, *C. flavipes* from the International Institute for Biological Control (IIBC) in Pakistan was imported to the IIBC station in Trinidad for establishment on *Diatraea saccharalis;* this became an important centre for the supply and distribution of *C. flavipes* in the New World (Potting 1996). Wasp populations from India were also exported to America and established on *D. saccharalis* in Barbados, the USA, Brazil, Colombia, Panama, Venezuela and Peru (Mohyuddin *et al.* 1981; Mecedo *et al.* 1993). More recently, the International Centre of Insect Physiology and Ecology (ICIPE) in Kenya initiated the releases of *C. flavipes* from Pakistan to several countries throughout east Africa. Thus, the close relationships and low genetic diversity found in this study among populations from the Asian subcontinent, Africa, North and South America and the Caribbean (Clade I) corroborate with the movement history of *C. flavipes* for biological control introductions in these regions. The relationships among haplotypes in Clade II may also be a direct result of species introductions. Greathead (1971) recorded introductions into Mauritius and Réunion from India in 1960, however an unconfirmed report by Breniere *et al.* (1985) stated that *C. flavipes* was introduced into Mauritius, Réunion and Madagascar in 1917 from Java. Walker (1994) found specimens deposited in The Natural History Museum, London, collected from Réunion in 1951, thus, *C. flavipes* was clearly present in this region before the introductions from India in the 1960's. Mohyuddin (1971) and Rajabalee & Govendasamy (1988) speculated that *C. flavipes* arrived in Mauritius from southeast Asia with its host *Chilo sacchariphagus*. Our results are consistent with this assumption, as populations from this region are closely related to populations from Indonesia and PNG. Other studies have found that the population from Mauritius exhibited differences from *C. flavipes* strains in morphology and biology. Kimani-Njogu

& Overholt (1997) found the morphology of the male genitalia from Mauritius different from other populations. Moreover, the Mauritius strain is unable to develop on the sympatric host *Sesamia calamistis* Hampson 1910 in sugarcane and maize (Rajabalee & Govendasamy 1988), whereas populations in Kenya have been found to successfully utilise this host (Ngi-Song *et al.* 1995). Further work investigating this variation in virulence in other members of clade II may prove interesting, as host suitability exerts a diversifying selection pressure that can be the basis for genetic divergence and indeed speciation (Roush 1990).

Genetic structure on a smaller geographic scale was found within *C. sesamiae* populations. The west Kenyan haplotype from Kitale exhibited a 2.5% sequence divergence to haplotypes from Mombasa in east Kenya and South Africa. This deep divergence is most likely due to the natural barrier of the Rift Valley, which plays an important role in shaping population structure of many invertebrate and vertebrate species in Kenya (Arctander *et al.* 1999; Pitra *et al.* 2002; Sezonlin *et al.* 2006). Local compatibility between parasitoid and host populations has also been found for *C. sesamiae* in Kenya. *Cotesia sesemiae* populations originating from western Kenya develop well on the native stem borer, *Busseola fusca* (Fuller 1901), however, populations from the coast and the Eastern Province, where *B. fusca* does not occur, cannot complete development in this host (Ngi-Song *et al.* 1995; Ngi-Song *et al.* 1998; Mochiah *et al.* 2001). A similar trend has been reported in Zimbabwe between *C. sesemiae* populations occurring in the highveld (> 1200 m) and the lowveld (< 600 m) regions (Chinwada *et al.* 2003). *Busseola fusca* populations were unsuitable hosts for lowveld *C. sesemiae*, but were suitable for development of the highveld population. Although the two populations were reproductively compatible, the authors attributed the differences in physiological compatibility to genetic variation between populations.

This study was intended to provide the geographic and historical context for understanding divergences within the *C. flavipes* complex. Genetic divergence amongst populations can be used as a framework for the detection of biological difference in host-parasitoid physiological compatibility and reproductive success. However, the evolution of biological differentiated strains cannot always be determined with mtDNA genetic structure. For instance, the Thailand population (clade I) exhibited low sequence divergence (0.3-1.1%) from other *C. flavipes* populations in Clade I. This contrasts with other studies that have demonstrated variability between Thailand populations and other populations of *C. flavipes*. Kimani-Njogu *et al.* (1998)

found that the Thailand strain was dissimilar to strains from Texas and Pakistan based on frequencies of 38 allozymes. In addition, biological divergence has also been found between the Thailand strain and other *C. flavipes* in morphology (Kimani-Njogu & Overholt 1997) and life history traits (Wiedenmann & Smith 1995). Likewise, low mitochondrial sequence divergence was found in the present study between populations from India/Pakistan and the New World, however there is some evidence that populations from these regions have differential reproductive success on a range of hosts. Potting *et al.* (1997b) investigated reproductive success among strains of *C. flavipes* from Pakistan, Texas and Thailand on *Chilo partellus* and the New World host, *Diatraea saccharalis*. All strains had a lower survival rate on *D. saccharalis* compared to the ancestral host, *C. partellus*. However, the Texas strain, which had the longest period of co-existence with the new host, had the highest survival rate on *D. saccaralis* compared to other tested strains, suggesting the existence of adaptive evolution.

The lack of mtDNA divergence in these examples suggests that the mtDNA data provides relatively little power to detect recent biological and host-related differentiation. Rapid evolution due to selection pressure may occur quickly with little time for lineage sorting to produce significant differences in neutral genetic markers (Avise 2000). Substitution rates in mtDNA evolution averages 2% per Myr in insects (Brower 1994). Although mtDNA evolution rates are increased in parasitic Hymenoptera due to the high level of radiation in this group (Castro *et al.* 2002), adaptive radiation can still be a more rapid process (Hartl & Clarke 1997; Reznick & Ghalambor 2001). Allopatric populations of the *C. flavipes* complex are coevolved with a unique suite of hosts in each geographic region and this selection to different hosts and environments can promote the rapid formation of adapted parasitoid strains and the emergence of incipient species (Roush 1990). Rapid adaptive evolution can also occur in biological control introductions when parasitoid species are introduced into a new environment with different host species. Nemec & Stary (1983) proposed that the level of heterozygosity displayed by a parasitoid species developing in different hosts would indicate the ancestral host for the species. The concept, referred to as the 'population diversity centre hypothesis', suggests that a parasitoid displays the highest level of heterozygosity in the ancestral host compared to subsequent host radiations. They demonstrated that several species of aphidiine braconids had a rapid loss of alleles when polymorphic strains were transferred from their original aphid host to alternate hosts, indicating that hosts exert

different selection pressures on different alleles (Nemec & Stary 1983). Therefore, parasitoid host switching events during biological control introductions may lead to the directional selection of some genotypes and a loss of heterozygosity, which can promote differences in the ability of a parasitoid to attack hosts within an expected host range (Powell & Wright 1992).

In conclusion, this is the first study to investigate the status of the *C. flavipes*-like species in Australia and the phylogeography of worldwide populations of the *C. flavipes* complex using DNA sequence data. Our results show that phylogenetic reconstructions based on mtDNA sequence data from two genes lead to similar interpretations of the intraspecific and interspecific relationships within the *C. flavipes* complex. The conclusions in this study can be used as a framework for investigating the ecological and adaptive differentiation of strains within the complex, information that can only be obtained by examining multiple neutral loci. Moreover, the reliance on mitochondrial DNA alone can give incorrect results due to selective sweeps on mitochondrial loci and mitochondrial introgression (Ballard & Whitlock 2004). Although the data corroborates the existence of a phylogeographic pattern that is associated with historical biogeographic barriers and the movement history of these species for biological control, the inclusion of nuclear markers would increase confidence in these results. Additional studies are also required to delineate species boundaries of Australian *Cotesia* haplotypes and to determine reproductive success of this taxon on high threat stem borer pests to the Australian sugar industry.

References

Alam M.M., Beg M.N., Ghani M.A. 1972. Introduction of *Apanteles* spp. against graminaceous borers into Pakistan. *Technical Bulletin, Commonwealth Institute Biological Control* 15:1-10.

Allsopp P., Samson P., Chandler K. 2000. Pest management, p. 436 *in:* Hogarth D. M. , Allsopp P. G. (eds.), *Manual of Canegrowing.* Brisbane.

Arctander P., Johansen C., Coutellec-Vreto M. 1999. Phylogeography of three closely related African bovids (tribe Alcelaphini). *Molecular Biology and Evolution* 16: 1724-1739.

Austin A.D., Dangerfield P.C. 1992. Synopsis of Australasian Microgastrinae (Hymenoptera: Braconidae), with a key to genera and description of new taxa. *Invertebrate Taxonomy* **6**: 1-76.

Avise J.C. 2000. *Phylogeography: the History and Formations of Species*. Harvard University Press, Cambridge, MA, 447 p.

Ballard J., Whitlock M. 2004. The incomplete natural history of mitochondria. *Molecular Ecology* **13**: 729-744.

Breniere J., Bordat D., Vercambre B., Hamza H., Renand M. 1985. Biological control operations against maize borer *Chilo partellus* (Swinhoe), Lepidoptera, in the island Ngazidga. *Agronomie Tropicale* **40**: 157-166.

Brower A. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius eratio* inferred from patterns of mitochondrial DNA evolution. *Proceeding of the National Academy of Sciences* **91**: 6491-6495.

Castro L., Austin A.D., Dowton M. 2002. Contrasting rates of mitochondrial molecular evolution in parasitoid Diptera and Hymenoptera. *Molecular Biology and Evolution* **19**: 1100-1113.

Chenna R., Sugawara H., Koike T., Lopez R., Gibson T.J., Higgins D.G., Thompson J.D. 2003. Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research* **31**: 3497–3500.

Chinwada P., Overholt W.A., Omwega C.O., Mueke J.M. 2003. Geographic differences in host acceptance and suitability of two *Cotesia sesamiae* populations in Zimbabwe. *Biological Control* **28**: 354-359.

Danforth B.N., Mitchell P.L., Packer L. 1998. Mitochondrial DNA differentiation between two cryptic *Halictus* (Hymenoptera: Halictidae) species. *Annals of the Entomological Society of America* **91**: 387-391.

Danks H.V. 1988. Systematics in support of entomology. *Annual Review of Entomology* **33**: 271-296.

Dowton M., Austin A.D. 1994. Molecular phylogeny of the insect order Hymenoptera: Aprocritan relationships. *Proceedings of the National Academic of Sciences USA* **91**: 9911-9915.

Dowton M., Austin A.D. 1995. Increased genetic diversity in mitochondrial genes is correlated with the evolution of parastism in the Hymenoptera. *Journal of Molecular Evolution* **41**: 958-965.

Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* **17**: 368-376.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.

FitzGibbon F., Allsopp P.G., De Barro P.J. 1999. Chomping, boring and sucking on our doorstep - the menace from the north. *Proceedings of the Australian Society of Sugar Cane Technologists* **21**: 149-155.

GentraSystems 2005. *DNA purification from a single fly*. Gentra Systems.

Greathead D.J. 1971. A review of biological control in the Ethiopean Region. *Technical Communication, Commonwealth Institute of Biological Control* **5**: 1-162.

Hall T.A. 1999. *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows*. North Carolina State University.

Hartl D.L., Clarke A.R. 1997. *Principles of Population genetics*. Sinauer, Sunderland, MA. 542 p.

Heimpel G.E., Antolin M.F., Franqui R.A., Strand M.R. 1997. Reproductive isolation and genetic variation between two „strains" of *Bracon hebetor* (Hymenoptera: Braconidae). *Biological Control* **9**: 149-156.

Huelsenbeck J.P., Ronquist F. 2001. *MrBayes 3. Bayesian Analysis of Phylogeny*. Uppsala University, San Diego, (http://mrbayes.csit.fsu. edu/index.php)

Hufbauer R.A., Bogdanowicz S.M., Harrison R.G. 2004. The population genetics of a biological control introduction: mitochondrial DNA and microsatellite variation in native and introduced populations of *Aphidus ervi*, a parasitoid wasp. *Molecular Ecology* **13**: 337-348.

Inayatullah C. 1983. Host selection by *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae): Influence of host and host plant. *Journal of Economic Enotomology* **76**: 1086-1087.

Ingram W.R. 1983. Biological control of graminaceous stem borers and pod-borers. *Insect Science and its Application* **4**: 205-209.

Jarvis E. 1927. *Notes on insects damaging sugar cane in Queensland*. Division of Entomology. Bureau of Sugar Experiment Stations, p. 9-11.

Kalshoven L.G.E. 1981. *Pests of Crops in Indonesia*. P T Ichtiar Baru-Van Hoeve, Jakarta, 701 p.

Kimani S.W., Overholt W.A. 1995. Biosystematics of the *Cotesia flavipes* complex (Hymenoptera: Braconidae): interspecific hybridization sex pheromone and mating behaviour studies. *Bulletin of Entomological Research* **85**: 379-386.

Kimani-Njogu S.W., Overholt W.A. 1997. Biosystematics of the *Cotesia flavipes* species complex (Hymenoptera: Braconidae), parasitoids of the gramineous stem borers. *Insect Science and its Application* **17**: 119-130.

Kimani-Njogu S.W., Overholt W.A., Woolley J., Walker A. 1997. Biosystematics of the *Cotesia flavipes* species complex (Hymenoptera: Braconidae): morphometrics of selected allopatric populations. *Bulletin of Entomological Research* **87**: 61-66.

Kimani-Njogu S.W., Overholt W.A., Woolley J., Omwega C.O. 1998. Electrophoretic and phylogenetic analyses of selected allopatric populations of the *Cotesia flavipes* complex (Hymenoptera: Braconidae), parasitoids of cereal stem borers. *Biochemical Systematics and Ecology* **26**: 285-296.

Kuniata L.S., Sweet C.P. 1994. Management of *Sesamia grisescens* Walker (Lep.:Noctuidae), a sugar-cane borer in Papua New Guinea. *Crop Protection* **13**: 488-493.

Lloyd P., Kuniata L.S. 2000. *The control of Sesamia at Ramu*. Sugarcane International, April, 10–17.

Mecedo N., de Araujo J.R., Botelho S.M. 1993. Sixteen years of biological control of *Diatraea saccharalis* (Fabr.) (Lepidoptera: Pyralidae) by *Cotesia flavipes* (Cam.) (Hymenoptera: Braconidae) in the state of Sao Paulo, Brazil. *Annual Society of Entomology-Brazil* **22**: 441-448.

Michel-Salzat A., Whitfield J.B. 2004. Preliminary evolutionary relationships within the parasitoid wasp genus *Cotesia* (Hymenoptera: Braconidae: Microgastrinae): combined analysis of four genes. *Systematic Entomology* **29**: 1-12.

Mochiah M.B., Ngi-Song A.J., Overholt W.A., Botchey M. 2001. Host suitability of four cereal stem borers (Lepidoptera: Crambidae, Noctuidae) for different geographic populations of *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) in Kenya. *Biological Control* **21**: 285-292.

Mohyuddin A.I. 1971. Comparative biology and ecology of *Apanteles flavipes* (Cam) and *A. sesamiae* Cam. as parasites of graminaceous borers. *Bulletin of Entomological Research* **61**: 33-39.

Mohyuddin A.I., Inayatullah C., King E.G. 1981. Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bulletin of Entomological Research* **71**: 575-581.

Nemec V., Stary P. 1983. Elpho-morph differentiation in *Aphidius ervi* Hal. biotype on *Microlophium carnosum* (Bckt.) related to parastization on *Acyrthosiphon pisum* (Harr.) (Hym., Aphidiidae). *Journal of Applied Entomology* **95**: 524-530.

Ngi-Song A.J., Overholt W.A., Ayertey J.N. 1995. Suitability of African graminaceous stem borers for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Environmental Entomology* **24**: 978-984.

Ngi-Song A.J., Overholt W.A., Stouthammer R. 1998. Suitability of *Busseola fusca* and *Sesamia calamistis* (Lepidoptera: Noctuidae) for the development of two population of *Cotesia sesamiae* (Hymenoptera: Braconidae) in Kenya. *Biological Control* **12**: 208-214.

Overholt W.A. 1998. *Biological Control*, p. 349-362 *in:* Polaszek A. (ed.), *African Cereal Stem Borers: Economic Importance, Taxonomy, Natural Enemies and Control.* CAB International, Wallingford.

Pitra C., Hansen J., Lieckfeldt D., Arctander P. 2002. An exceptional case of historical outbreeding in African sable antelope populations. *Molecular Ecology* 11: 1197-1208.

Polaszek A., Walker A.K. 1991. The *Cotesia flavipes* species-complex: parasitoids of cereal stem borers in the tropics. *Redia* 74:335-341.

Posada D., Crandall K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Potting R.P.J., Otten H., Vet L.E.M. 1997a. The relation between parasitoid ecology and learning: absence of learning in the stem borer parasitoid *Cotesia flavipes*. *Animal Behaviour.* 53: 1211-1223.

Potting R.P.J., Vet L.E.M., Overholt W.A. 1997b. Geographic variation in host selection behaviour and reproductive success in the stem borer parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae). *Bulletin of Entomological Research* 87: 515-524.

Potting R.P.J. 1996. *Hunting for the hiding hosts: the behavioural ecology of the stem borer parasitoid Cotesia flaivpes.* Ph.D. Dissertation, Wageningen Agricultural University 125 p.

Powell W., Wright A.F. 1992. The influence of host food plants on host recognition by four Aphidiine parasitoids (Hymenoptera: Braconidae). *Bulletin of Entomological Research* 81: 449-453.

Rajabalee M.A., Govendasamy M. 1988. Host specificity and efficacy of *Apanteles flavipes* (Cam.) and *A. sesamiae* (Cam.) (Hymenoptera: Braconidae) parasites of sugarcane moth borers in Mauritius. *Revue Agricole et Sucrière* 67: 78-80.

Reznick D., Ghalambor C. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* 112-113: 183-198.

Roush R.T. 1990. Genetic variation in natural enemies: critical issues for colonization in biological control, *in:* Mackauer M., Ehler L.E., Roland J. (eds.), *Critical Issues in Biological Control.* Intercept Ltd, Andover, Hants.

Sallam M.N., Allsopp P.G. 2002. Preparedness for borer incursion into Australia. *Australian Sugarcane* 5: 5-7.

Schauff M.E., LaSalle J. 1998. The relevance of systematics to biological control: protecting the investment in research. *Pest Management - Future Challenges. Proceeding of the Sixth Australasian Applied Entomological Research Conference* 1: 560.

Sezonlin M., Dupas S., Le Rü B., Le Gall P., Moyal P., Calatayud P.-A., Giffard I., Faure N., Silvain J.-F. 2006. Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (lepidoptera, Noctuidae) in sub-Saharan Africa. *Molecular Ecology* 15: 407-420.

Simon C., Francesco F., Beckenbach A., Crespi B., Liu H., Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701.

Smith P.T., Kambhampati S. 1999. Status of the *Cotesia flavipes* species complex (Braconidae: Microgastrinae) based on mitochondrial 16S, rRNA and NADH 1 dehydrogenase gene sequence. *Journal of the Kansas Entomological Society* 72: 306-314.

Swofford D.L. 2000. *PAUP* Phylogenetic Analysis Using Parsimony - and other methods.* Sinauer Associates, Sunderland.

Walker A.K. 1994. Species of Microgastrinae (Hymenoptera: Braconidae) parasitizing lepidopterous cereal stem borers in Africa. *Bulletin of Entomological Research* 84: 421-434.

Watanabe C. 1932. A revision of braconid-species parasitic in the injurious insects of rice-plant and sugar-cane in Japan and Formosa. *Transactions of the Sapporo Natural History Society* 12: 63-72.

Watanabe C. 1965. Notes on the *Apanteles flavipes* complex (Hymenoptera: Braconidae). *Mushi* 38: 111-116.

Whitfield J.B. 1997. Molecular and morphological data suggest a common origin for the polydnaviruses among braconid wasps. *Naturwissenschaften* 84: 502-507.

Whitfield J.B., Cameron C.M. 1998. Hierarchical analysis of variation in the mitochondrial 16S rRNA gene among Hymenoptera. *Molecular Biology and Evolution* 15: 1728-1743.

Wiedenmann R.N., Smith J.W.J. 1995. Parasitization of *Diatraea saccharalis* by *Cotesia chilonis* and *Cotesia flavipes*. *Environmental Entomology* 24: 950-961.

Wilkinson D.S. 1928. A revision of the Indo-Australian species of the genus *Apanteles* (Hymenoptera: Braconidae). *Bulletin of Entomological Research* 19: 79-105.

Young G.R., Kuniata L.S. 1992. Life history and biology of *Sesamia grisescens* Walker (Lepidoptera: Noctuidae), a sugarcane borer in Papua New Guinea. *Journal of the Australian Entomological Society* 31: 199-203.

——————————————————— ARTICLE

Single-step PCR differentiation of *Cotesia sesamiae* (Cameron) and *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) using polydnavirus markers

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Abstract. *Cotesia sesamiae* (Cameron) and *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) are the main larval parasitoids of cereal stemborers in sub-Saharan Africa. *Cotesia sesamiae* is endemic to eastern and southern Africa, while *C. flavipes* was introduced into the region for biological control against the exotic lepidopteran *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). The two are sibling parasitoids, difficult to distinguish morphologically. The introduced insect could potentially lead its African biotype to extinction because of their similar ecological niche. In order to distinguish the two species, multiplex primer-specific and PCR-RFLP tests were developed. Rapid identification of the two species was possible using primer-specific tests on DNA extracts as well as on pieces of tissue in a single PCR step followed by gel electrophoresis. The CRV1 gene of the polydnavirus, a symbiont to the wasps, was used as the marker. The results show that the morphological identifications, validated by molecular tests, are accurate in 93% of cases.

Résumé. Simple analyse par PCR pour distinguer *Cotesia sesamiae* (Cameron) (Hymenoptera : Braconidae) de *Cotesia flavipes* Cameron (Hymenoptera : Braconidae) en utilisant des marqueurs du polydnavirus. *Cotesia sesamiae* (Cameron) et *C. flavipes* Cameron (Hymenoptera : Braconidae) sont parmi les principaux parasitoïdes larvaires des foreurs de tige de céréales cultivées en Afrique Sub-Saharienne. L'un est endémique, l'autre fut introduit à des fins de lutte biologique contre un lépidoptère exotique foreur de tige, *Chilo partellus* (Swinhoe). Les deux parasitoïdes sont difficilement distinguables morphologiquement. *Cotesia flavipes* présente une niche écologique similaire à l'un des biotypes de *C. sesamiae*. Il peut potentiellement le menacer d'extinction. Afin de distinguer les espèces à l'aide de tests fiables et de mieux suivre l'évolution de leurs effectifs, nous avons développé un test PCR-RFLP et un test multiplex-amorce spécifique. Le test amorce-spécifique a permis l'identification rapide des deux espèces à partir d'ADN extrait ou de morceaux de tissu en une seule étape de PCR suivie d'une électrophorèse sur gel. Les résultats montrent que les identifications morphologiques ne sont correctes que dans 93% des cas.

Keywords: Species differentiation, *Cotesia flavipes*, *Cotesia sesamiae*, multiplex PCR, polydnavirus.

Classical biological control aims to stabilize ecosystems that have been destabilized by the introduction of exotic invaders or by intensive agriculture. It is based on the beliefs that the invaders' ecological success (usually, the pests' ecological success) is due, at least partly, to the absence of natural enemies in the environment, and that the introduction of natural enemies will allow the system to return to equilibrium. However the biological control agent is itself an invader in the system, and can be unsafe for the ecosystem also (Holt & Hochberg 2001; Hopper 2001; Waage 2001). In many cases, antagonist species closely related to the introduced agent are present and are difficult to distinguish morphologically (Perdikis

et al. 2003). In order to follow up introductions and better evaluate the effect of biological control on the natural endemic fauna of antagonists, it is necessary to develop rapid taxonomic diagnostic tools (Hinz *et al.* 2001).

Cotesia sesamiae (Cameron 1891) and *Cotesia flavipes* Cameron 1891 (Hymenoptera: Braconidae) are the main larval parasitoids of cereal stemborers in eastern and southern Africa. *Cotesia sesamiae* is endemic to Africa and *C. flavipes,* a native to Asia, has been introduced repeatedly in Africa since 1993 as a biological control agent to control the exotic stemborer *Chilo partellus* (Swinhoe 1885) (Lepidoptera: Crambidae) (abbreviated as *Ch. partellus* in this paper). The pest originates from India but was introduced into Africa in the 1930s. *Cotesia flavipes* is now widespread over eastern and southern Africa. Both parasitoids have similar ecological niches. *Cotesia sesamiae* exists

in two biotypes, one of which is virulent to *Busseola fusca* (Fuller 1901) (Lepidoptera: Noctuidae), while the other, like *C. flavipes,* is avirulent and unable to develop on *B. fusca* (Ngi-Song *et al.* 1998). On *Ch. partellus,* the intrinsic rate of population increase of *C. flavipes* was higher than *C. sesamiae* at all temperatures, suggesting that the exotic parasitoid is able to respond faster to host density changes (Mbapila 1994). Due to the higher rate of parasitism of *C. flavipes* on *Ch. partellus,* there is the possibility that *C. flavipes* might drive the avirulent biotype of *C. sesamiae* to extinction in areas where *B. fusca* is absent. On the other hand, it has been observed that *C. flavipes* is attracted more strongly to infested maize, while *C. sesamiae* exhibits a preference for infested sorghum (Ngi-Song *et al.* 1996); these differences may prevent competitive displacement. This entomological risk is a question that needs to be well documented and understood for the sustainability of biological control programmes (Hopper 2001; Waage 2001). The exotic *C. flavipes* is now the dominant parasitoid in the coastal belt of Kenya where *Ch. partellus* is dominant (Zhou *et al.* 2003), but no evidence from the field was found of the competitive exclusion of *C. sesamiae* by *C. flavipes* (Sallam *et al.* 2001). Since it is expected that the rate of parasitism will depend on the prevalence on each plant and stemborer species, field data are needed on the abundance of *C. flavipes* and *C. sesamiae* under these different ecological conditions.

One difficulty in these surveys is that the two species are very difficult to distinguish morphologically (Kimani-Njogu & Overholt 1997). A fast diagnostic test is needed.

Previous studies have shown that infrared spectroscopy can distinguish the cocoons of the two species with an accuracy of more than 85% (Cole *et al.* 2003). Molecular markers may also be of use. Polymerase chain reaction (PCR) amplification is a powerful technique for species diagnosis that has been used for identifying pathogens and parasites, and for distinguishing between sibling species of macroscopic, free-living organisms. The best known technique, PCR-RFLP, is based on specific digestion of a diagnostic amplimer. It is highly reliable and therefore widely used, especially with the development of barcoding systematics (Blaxter *et al.* 2005). Another technique, based on allele-specific PCR and primer-induced fragment-length variation, appears more cost effective and powerful. It uses one forward primer that is common to all targets and one reverse specific primer that anneals at different positions for each target. The amplimers are separated directly by gel electrophoresis. Direct amplification from pieces of tissue is also used

to accelerate the technique and reduce its cost.

Polydnaviruses are obligatory symbionts of many braconid and ichneumonid parasitoid wasps. In braconids, symbiosis is dated at ca. 73 million years ago and involves around 17 500 species of the microgastroid complex. The virus is transmitted from one generation to the next on wasp chromosomes; no horizontal transmission has been detected so far. The perfect parallel phylogeny observed between wasp and polydnavirus genes is consistent with the premise that these symbionts can be considered as other nuclear genes (Whitfield 2000). Nevertheless, molecular investigations suggest that the rate of substitution of polydnavirus genes is accelerated by positive Darwinian selection and by increased mutation rates compared to other nuclear genes (Dupas *et al.* 2003). This makes them suitable markers at low phylogenetic levels, within species, or especially for distinguishing between closely related species. In addition, the polydnavirus markers are pre-amplified in the wasps' ovaries through a virus replication process, which makes them easier to detect by PCR.

Cotesia rubecula (Marshall 1885) (Hymenoptera: Braconidae) CrV1 is a polydnavirus gene involved in host immune suppression by targeting host haemocytes. The aim of this work was to develop a PCR test for the identification of the two species of stemborer parasitoids based on sequence differences in the polydnavirus gene CrV1 between *C. sesamiae* and *C. flavipes.* Two techniques were used, one based on PCR-RFLP and one based on primer-specific direct PCR fragment-length differences.

Material and Methods

Insect sampling

Specimens originating from 97 localities in sub-Saharan Africa (Kenya, Tanzania, Congo) and three localities in Asia (India and Pakistan) were used in the analyses. Some individuals were sequenced for the CrV1 marker in order to develop a molecular test; others were used directly for the PCR tests. The eight *C. flavipes* individuals sequenced came from India (1 locality), north and south Pakistan (1 locality each) and Kenya (5 localities); the 18 *C. sesamiae* used for sequencing were from Congo (3 localities), Kenya (12 localities) and Tanzania (3 localities). Additional individuals were used for the PCR tests. A total of 28 *C. flavipes* from 17 localities in Kenya, and 205 *C. sesamiae* from 84 localities across sub-Saharan Africa (16 localities in Congo; 65 localities in Kenya; 3 localities in Tanzania) were genotyped either by sequencing, by PCR tests or both.

Morphological identification

Morphological identification was performed on one or two males per cocoon mass, based on the morphology of the genitalia (Kimani-Njogu & Overholt 1997).

Molecular characterization

Whole insect bodies were frozen in liquid nitrogen for 1 min and ground in a mortar and pestle. Different extraction protocols were used for the sequencing and PCR tests. For sequencing, total DNA was extracted using a DNEasy tissue Kit (Qiagen Gmbh). For the PCR tests, individuals were extracted using the Chelex® (BioRad) protocol, or the PCR was performed directly on non-crushed bodies or on the last three to four abdominal segments. For Chelex extraction, 100 µl 5% Chelex was added to the ground insect and mixed with a mortar and pestle. Protein-Chelex adhesion was performed during two cycles of 10 min each at 99 °C and separation was done by three tube inversions at room temperature.

For sequencing, a 999 bp fragment of the CrV1 polydnavirus gene was amplified using primers CrV1087F: 5'ATGTCACTCGTCAAAAGTGC3' and CrV2107R: 5'AAAGTTTGCGATGGGGTTGT3' designed from the C. rubecula CrV1 gene sequence and named according to their positions in the GeneBank sequence AF359344. Another set of primers CsfV1125F: 5'TCTCCTGTGTCAATCATGTAAGTT3', and CsfV1955R: 5'ACTCCTTCAACGCTGGGTTCCTTG3', were designed from the C. sesamiae and C. flavipes sequences obtained in the present study. The PCR cycling conditions were as follows: initial denaturation 5 min at 94 °C, 40 cycles of 50 s at 94 °C, 1 min 20 s at 51 °C (52 °C for CsfV1125F-CsfV1955R primer pair) and 1 min 20 s at 72 °C. Final extention was 10 min at 72 °C. The reaction mixture contained 0.4 µM primers, 0.24 µM dNTP, and 1µl DNA plus 1 Taq T4 DNA polymerase (Promega) per 25 µl of reaction. The MgCl$_2$ concentration was 2 mM for the CrV1087-CrV2109 primer pair and 1.5 mM MgCl$_2$ for the CsfV1125F-CsfV1955R primer pair. Sequencing reactions were performed in both directions using the amplification primers on an automated sequencer (ABI PRISM 3100).

A primer-specific direct PCR test was developed based on the CrV1 sequences, with two forward primers designed to anneal specifically to C. sesamiae and C. flavipes sequences at different positions and one reverse annealing to both (fig. 1). The C. sesamiae-specific primer was CsV1394F 5'AACGAACACTTTCGATGAA3' and the C. flavipes-specific primer was CfV1634F 5'GAGTATTTTCCGAAAATGG3'. The reverse, non-specific primer was Csf1955R. Expected amplimer sizes based on C. rubecula sequences were 511 bp and 271 bp for C. sesamiae and C. flavipes, respectively. PCR cycling was the same as above except for the annealing temperature, at 60 °C. The reaction mixture contained 0.28 µM primers, 0.24 µM dNTP, and 0.5 µl DNA plus 0.5u Promega® Taq DNA polymerase per 12.5 µl of reaction. Reactions were performed either on Chelex extracts or directly on non-extracted tissues. For each PCR reaction, a negative control (water) and positive controls (confirmed C. flavipes and C. sesamiae DNA extracts) were performed. Amplimers were loaded on a 1% agarose-0.5% TBE gel next to Invitrogen® 100bp ladder. Migration was performed in 0.5% TBE during 30 min at 100V on an i-mupid® (Eurogentec) gel migration system.

Repeatability and discrepancies between morphological and molecular identifications

When different individuals from the same cocoon mass are used for the morphological and molecular identification, any discrepancies may be due to multiparisitism by C. flavipes and C. sesamiae (i.e. two species in the same cocoon mass) (Ngi-Song et al. 2001). Therefore, in the present study when molecular and morphological identifications were different, other individuals of the cocoons were tested by PCR to confirm that the cocoon mass did not contain a species mix of C. flavipes and
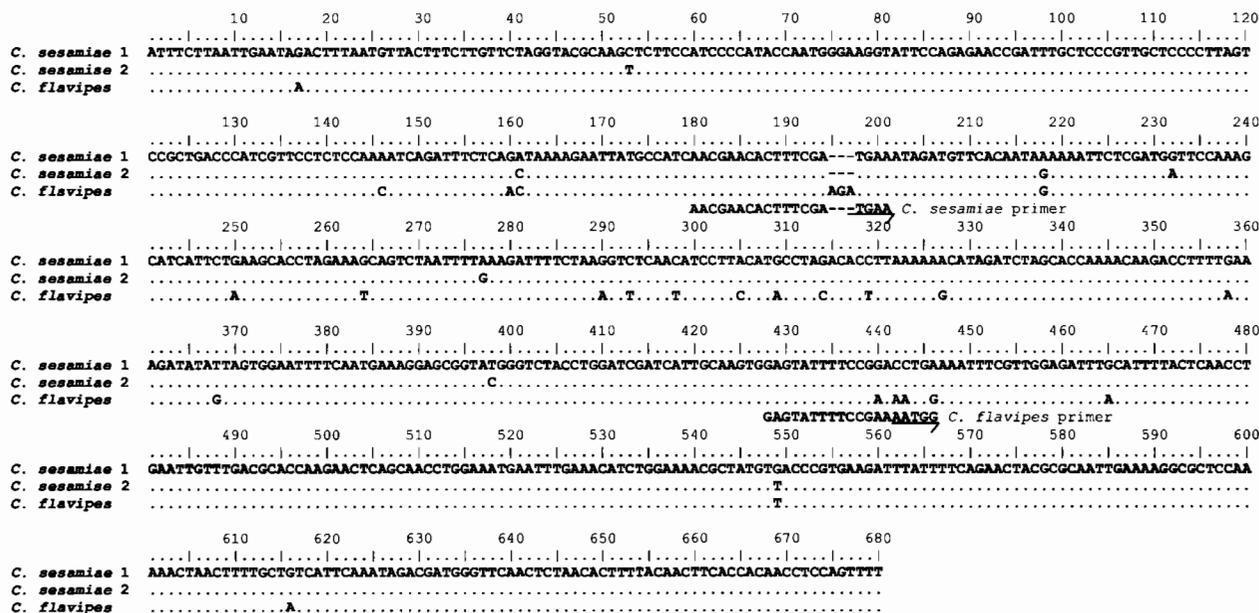

Figure 1
Cotesia sesamiae and *Cotesia flavipes* polydnavirus CrV1 haplotypes, and species-specific primers.

C. sesamiae. In addition, the Cytochrome *b* mitochondrial gene was sequenced on six of the individuals showing discrepancies (P89 Kisumu 1, P172 Rift Valley 3, P77 Mt Kenya 1, P210 Mt Kenya 2, P80 Mt Kenya 3, and P 162 Mombasa 6, all from Kenya). The primers were CB1 and CB2 (Simon *et al.* 1994). PCR annealing was done at 48°C with 3 mM MgCl$_2$. All other conditions were as for CrV1.

A PCR test was also developed based on CrV1 sequences. Enzyme Fok I was found to cut the CsfV1125F-CsfV1955R amplimer once at position 76 for *C. flavipes* and twice, at positions 76 and 374, for *C. sesamiae.* Expected fragment sizes based on the sequences were 76 bp, 298 bp and 456 bp for *C. sesamiae* and 76 bp and 754 bp for *C. flavipes.* This test was developed as an alternative to the primer-specific test, but was not used in this study.

Results

A fragment of 680 bp encoding CrV1 was sequenced from eight *C. flavipes* and 19 *C. sesamiae* individuals from one locality each, over their entire natural distribution range. We observed three haplotypes, one in *C. flavipes* and two in *C. sesamiae* (GeneBank accession numbers DQ356254 to DQ356256) (fig. 1). The divergence was 1.0% between *C. sesamiae* haplotypes and 3.5% and 3.7% between the *C. flavipes* haplotype and the two *C. sesamiae* haplotypes. Based on this sequence survey, diagnostic sequence variations were selected to distinguish the two species (fig. 1). A primer-specific test was developed. PCR amplification led to one band around 600 bp for *C. flavipes*, or one band around 300 bp for *C. sesamiae*, as expected from the sequencing data.

We investigated 233 cocoon masses for discrepancy between mophological and molecular identifications, of which 28 had been attributed to *C. flavipes* (noted *Cf* in this paragraph) morphologically and 205 to *C.*

sesamiae (*Cs*). The type PCR gel obtained is presented in fig. 2. Among the 28 individuals identified as *Cf* morphologically, 25 were *Cf* and 3 were *Cs* based on the PCR tests. Among the 205 morphological-*Cs*, 192 were *Cs* and 13 were actually *Cf* based on the PCR test (tab. 1). Some of the cocoon masses identified morphologically as *Cs* but characterized as *Cf* with the PCR test were analysed in more detail. Other cocoons were taken from the cocoon mass and run for PCR tests. Two of the eight cocoon masses were shown to be a *Cf* + *Cs* species mix, and the remaining six proved to be pure *Cf* (tests were performed on 3 to 7 additional individuals from the cocoon masses showing discrepancies) by PCR. The reliability of the PCR test on CrV1 was confirmed by sequencing the Cytochrome *b* fragment of six individuals (see Material and Methods) that had been identified as *Cs* morphologically but as *Cf* on PCR tests. All were confirmed as *Cf* based on the Cytochrome b sequence (GeneBank DQ459001, Dupas unpublished data).

Some PCR tests were also performed on pieces of tissue without DNA extraction. The results, presented in fig. 2, show that the reaction can be performed effectively on abdomen ends, cut from either alcohol-preserved or dried specimens, but not as satisfactorily on non-crushed whole bodies. Since we were confident of the molecular tests, the rate of morphological misidentification was calculated based on the results of the molecular tests. Among the entire sample tested, *Cf* was more likely to be considered morphologically as *Cs* than *vice versa;* 13 of the 37 individuals genotyped as *Cf* had been identified morphologically as *Cs* (35.1 % error), while only three of the 195 genotyped *Cs* individuals had been identified as *Cf* morphologically
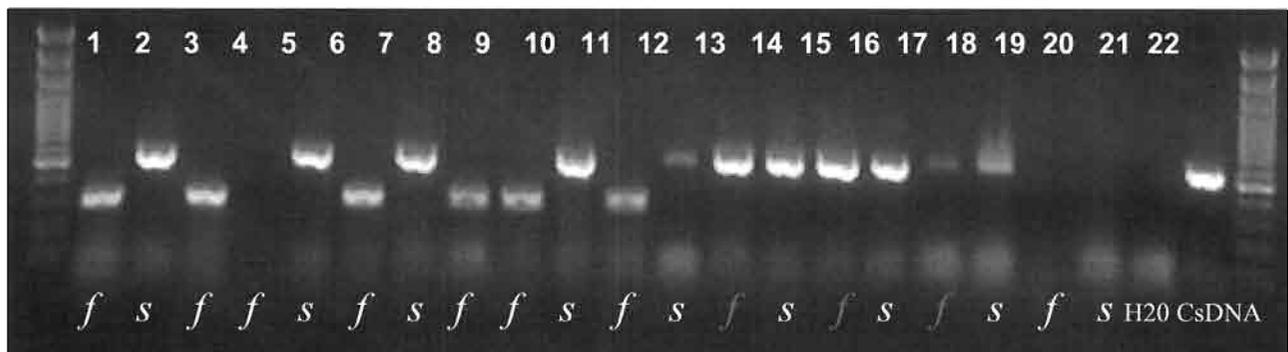
Figure 2
Results of PCR test performed on non-extracted DNA material (lanes 1-20): cut abdomen ends (lanes 1-16) or whole body (lanes 17-20). Individual specimens were either preserved in fresh alcohol (lanes 1-7), dried within the previous month (lanes 8-12), alcohol-preserved for 3 years (lanes 13-14), dried for 3 years (lanes 15-18), or freshly dried (lanes 19-20). Lane 21, negative control. Lane 22, *Cs* DNA extract positive control. "*s*" was identified as *Cotesia sesamiae* and "*f*" identified as *Cotesia flavipes* morphologically. The *C. sesamiae* band co-migrates with the 500 bp length marker, and the *C. flavipes* band with the 300 bp length marker. Samples for which molecular identification differs from morphological identification are indicated in grey.

(1.5% error). Overall, the reliability of morphological identification was 93.1%.

Discussion

A PCR test based on species-specific CrV1 polydnavirus gene primers was designed to distinguish between the sibling species *C. flavipes* and *C. sesamiae*. The results showed the reliability and rapidity of this method. The test consists of a single PCR followed by gel electrophoresis visualisation using a mix of three primers and performed on extracted DNA or even non-extracted pieces of tissues from dried or alcohol-preserved specimens of either sex. The viral nature of the genes and their localization in the calyx of the female increases the number of copies and may improve the successful application of PCR amplification, allowing for a fast, single-tube extraction PCR procedure, even from non-extracted abdomen ends.

The PCR tests developed here are based on a survey of *C. sesamiae* and *C. flavipes* sequences present in central and eastern sub-Saharan Africa. The survey did not include West African *C. sesamiae*. Although *C. sesamiae* is rarely found in that region, it remains to be confirmed whether the PCR test can be used for insects collected there. The *C. flavipes* populations included in the analyses were either from sub-Saharan Africa or from the insects collected in India and Pakistan for release in eastern and southern Africa. The reliability of the PCR tests in differentiating the two species remains to be checked for other regions.

Molecular tests showed that the insects had been correctly identified from male genitalia criteria with a 93.1% reliability. Morphological identification may be good enough in many situations, but molecular testing may be useful to estimate the morphological identification bias toward one or the other species in post-release surveys.

References

Blaxter M., Mann J., Chapman T., Thomas F., Whitton C., Floyd R., Abebe E. 2005. Defining operational taxonomic units using DNA barcode data. *Philosophical Transactions: Biological Sciences* **360**: 1935-1943.

Cole T.J., Ram M.S., Dowell F.E., Omwega C.O., Overholt W.A., Ramaswamy S.B. 2003. Near-infrared spectroscopic method to identify *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Annals of the Entomological Society of America.* **96**: 865-869.

Dupas S., Carton, Y., Poirié M. 2003. The genetic dimension of the coevolution of virulence resistance in *Drosophila* - parasitoid wasp relationships. *Heredity* **90**: 84-89.

Hinz W.E., Becker E.M., Shamoun S.H. 2001. Development of genetic markers for risk assessment of biological control agents. *Canadian Journal of Plant Pathology* **23**: 13-18.

Holt R.D., Hochberg M. E. 2001. Indirect interactions, community modules, and biological control: a theoretical perspective, p. 13-37 *in*: Wajnberg E., Scott J.K., Quimby P.C. (eds.), *Evaluating indirect ecological effects of biological control.* CAB International.

Hopper K.R. 2001. Research needs concerning non-target impacts. of biological control introductions, p. 39–56 *in*: Wajnberg E., Scott J.K., Quimby P.C. (eds.), *Evaluating indirect ecological effects of biological control.* CAB International.

Kimani-Njogu S.W., Overholt W.A. 1997. Biosystematics of the *Cotesia flavipes* species complex (Hymenoptera: Braconidae), parasitoids of the gramineous stem borers. *Insect Science and its Application* **17**: 119-130.

Mbapila J. 1994. Laboratory life tables of *Cotesia* spp. reared at difference temperatures on *Chilo partellus*, p31 *in*: *1994 ICIPE Annual Report.* ICIPE Science Press, Nairobi

Ngi-Song A.J., Overholt W.A., Njagi P.G.N., Dicke M., Ayertey J.A., Lwande W. 1996. Volatile infochemicals used in host and host habitat location by *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), larval parasitoids of stem borers on graminae. *Journal of Chemical Ecology* **22**: 307-323.

Ngi-Song A.J., Overholt A.W., Stouthamer R. 1998. Suitability of *Busseola fusca* and *Sesamia calamistis* (Lepidoptera: Noctuidae) for the development of two populations of *Cotesia sesamiae* (Hymenoptera: Braconidae) in Kenya. *Biological Control* **12**: 208-214.

Ngi-Song A.J., Kimani-Njogu S.W., Overholt W.A. 2001. Multiple parasitism by *Cotesia sesamiae* and *Cotesia flavipes* (Hymenoptera: Braconidae) on *Busseola fusca* (Lepidoptera: Noctuidae). *Biocontrol Science and Technology* **11**: 381-390.

Perdikis D.C., Margaritopoulos J.T., Stamatis C., Mamuris Z., Lykouressis D., Pekas J.A.T. 2003. Discrimination of the closely related biocontrol agents *Macrolophus melanotoma* (Hemiptera: Miridae) and *M. pygmaeus* using mitochondrial DNA analysis. *Bulletin of Entomological Research* **93**: 507-514.

Sallam M.N., Overholt W.A., Zhou G. 2001. Will *Cotesia flavipes* drive its African homologue, *Cotesia sesamiae*, to extinction? *Insect Science and its Application* **21**: 297-302.

Simon, C. Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences: a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**: 651-701.

Waage J.K. 2001. Indirect ecological effects of biological control: the challenge and the opportunity, p. 1-12 *in*: Wajnberg E., Scott J.K., Quimby P.C. (eds.), *Evaluating indirect ecological effects of biological control.* CAB International.

Whitfield J.B. 2000. Phylogeny of microgastroid braconid wasps, and what it tells us about polydnavirus evolution, p. 97-105 *in*: Austin A.D., Dowton M. (eds.), *The Hymenoptera: Evolution, biodiversity and biological control.* CSIRO Publishing, Melbourne.

Zhou G. Overholt W.A., Kimani-Njogu S.W. 2003. Species richness and parasitism in an assemblage of parasitoids attacking maize stem borers in coastal Kenya. *Ecological Entomology* **28**: 109-118.

　　　　　　　　　　　　　　　　　　　　　　　ARTICLE

Genetic Diversity of *Sturmiopsis parasitica* Curran (Diptera: Tachinidae)

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Abstract. *Sturmiopsis parasitica* Curran (Diptera: Tachinidae) is a widely spread parasitoid of various lepidopteran stem borers including *Eldana saccharina* Walker (Lepidoptera: Pyralidae), *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) in western, eastern and southern Africa. As the African sugarcane stalk borer, *E. saccharina*, is currently the most economically important insect pest in South African sugarcane there is an urgent need to develop a control strategy for the management of this pest. *S. parasitica* has been tested as a biological control agent against *E. saccharina* with limited success. In seeking possible reasons for this limited success we tested whether there is genetic differentiation among populations of *S. parasitica* and whether there are host-associated lineages of *S. parasitica* from *B. fusca* and *E. saccharina*.
To assess these hypotheses, DNA sequences of cytochrome oxidase I (COI), a mitochondrial protein-coding gene, were obtained from fifteen specimens collected in western, eastern and southern Africa. Phylogenetic analysis of these sequences using maximum parsimony grouped the specimens into two main clades, one of which is further subdivided. Examination of pairwise sequence divergence levels supports the hypothesis of two cryptic lineages. However, further supportive evidence is necessary before revising the taxonomy of the species.

Résumé. Diversité génétique de *Sturmiopsis parasitica* Curran (Diptera: Tachinidae). *Sturmiopsis parasitica* Curran (Diptera: Tachinidae) est un parasitoïde très répandu de plusieurs espèces de lépidoptères foreurs de graminées comme *Eldana saccharina* Walker (Lepidoptera: Pyralidae), *Busseola fusca* (Fuller) et *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) en Afrique sub-saharienne. Puisque *E. saccharina* est actuellement considéré comme un ravageur causant d'importants dommages sur canne à sucre en Afrique du Sud, il est apparu urgent de développer une stratégie de lutte biologique pour le contrôle de cet insecte. L'utilisation de *S. parasitica* en tant qu'agent de lutte biologique contre *E. saccharina* s'est avérée peu fructueuse. On pourrait faire l'hypothèse que ce parasitoïde généraliste, capable de parasiter de nombreuses espèces, présente une structuration génétique liée à l'hôte.

Dans ce contexte, le séquençage d'un fragment du gène mitochondrial codant pour la cytochrome oxydase I a été entrepris à partir de différents spécimens de *S. parasitica* provenant d'Afrique de l'Ouest, de l'Est et du Sud. Une analyse phylogénétique a permis de séparer les spécimens analysés en deux clades majeurs. L'analyse des distances génétiques entre haplotypes a confirmé l'existence de deux lignées cryptiques. D'autres études s'avèrent nécessaires avant de réviser la taxonomie de cet insecte parasitoïde.

Keywords: *Sturmiopsis parasitica*, mitochondrial DNA, biotype, molecular systematics, *Eldana saccharina*.

The genus *Sturmiopsis* Townsend 1916 (Diptera: Tachinidae) contains one Afrotropical species (*S. parasitica* Curran 1939), one Oriental species (*S. inferens* Townsend 1916) and one Palaearctic species (*S. emdeni* Mesnil 1959) known only from Israel (Barraclough 2004). *S. parasitica* is widespread in eastern, western and southern Africa, the type locality being Harare, Zimbabwe, but *S. parasitica* has

also been recorded from Burkina Faso, Cape Verde, Ghana, Ivory Coast, Kenya, Malawi, Niger, Nigeria, Senegal and Tanzania (Conlong 1997). *S. parasitica* is a widely spread parasitoid of various lepidopteran stem borers including *Eldana saccharina* Walker 1865 (Lepidoptera: Pyralidae), *Busseola fusca* (Fuller 1901) (Lepidoptera: Noctuidae) and *Sesamia calamistis* Hampson 1910 (Lepidoptera: Noctuidae) in western, eastern and southern Africa (Conlong 2000; Chinwada *et al.* 2004). Since *S. parasitica* occurs in sugarcane, maize and sorghum, its potential as a biocontrol agent is considered to be high (Nagarkatti & Rao 1975).

As *E. saccharina* is currently the most economically

important insect pest in South African sugarcane there is an urgent need to develop a control strategy for the management of this pest. Reliable laboratory rearing techniques have been developed for *S. parasitica*, and in laboratory experiments *S. parasitica* has shown much promise at locating *E. saccharina* in sugarcane grown in pots. It is now very important to start assessing its impact on *E. saccharina* under open field conditions in the South African sugarcane belt (Conlong 1997), as parasitised *E. saccharina* larvae have been recovered from release sites in KwaZulu-Natal indicating the potential of *S. parasitica* as a biological control agent against *E. saccharina* (Martin 1999).

The following questions however arise: is there genetic differentiation among populations of *S. parasitica* in its geographic areas of distribution, and are there a host-associated lineage in *S. parasitica* from *B. fusca* and *E. saccharina*?

Material and methods

Taxon sampling

Seventeen specimens were sampled (Table 1), including seven *S. parasitica* from Benin, one from Senegal, two from Kenya, five from Zimbabwe and two specimens of *S. inferens* from Bangalore, India, the latter included as an outgroup.

Laboratory techniques

DNA was extracted from individual specimens using the Qiagen DNeasy™ Tissue Kit. Polymerase chain reaction (PCR) was accomplished using the primers C1-J-1514 (5'GGTCAACAAATCATAAAGATATTGG3') and C1-N-2173 (5'TAAACTTCAGGGTGACCAAAAAATCA3') (Simon *et al.* 1994). PCR amplifications were performed on a Perkin Elmer GeneAmp PCR System 2400 under the following conditions: 95 °C for 10 minutes, 35 cycles of (94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 2 minutes), 72 °C for 7 minutes, 4 °C hold. Each 50 µl PCR reaction mix contained 1X Supertherm Gold PCR Buffer (JMR Holdings,

Table 1. Localities and hosts from which *Sturmiopsis* specimens were sampled.

Haplo-type	Species	DNA no.	Country	Locality		Latitude, Longitude	Host Insect	Host Plant
-	*S. inferens*	533	India	Coimbatore	Tamil Nadu State	11°01'N, 76°96'E	*Chilo infuscatellus*	Sugarcane
-	*S. inferens*	534	India	Coimbatore	Tamil Nadu State	11°01'N, 76°96'E	*Chilo infuscatellus*	Sugarcane
HT: A	*S. parasitica*	384	Benin	Calavi, Cotonou	International Institute of Tropical Agriculture	6°25'N, 2° 20'E	*Eldana saccharina*	Maize
HT: A	*S. parasitica*	385	Benin	Calavi, Cotonou	International Institute of Tropical Agriculture	6°25'N, 2° 20'E	*Eldana saccharina*	Maize
HT: A	*S. parasitica*	431	Benin	Calavi, Cotonou	International Institute of Tropical Agriculture	6°25'N, 2° 20'E	*Eldana saccharina*	Maize
HT: A	*S. parasitica*	432	Benin	Calavi, Cotonou	International Institute of Tropical Agriculture	6°25'N, 2° 20'E	*Eldana saccharina*	Maize
HT: A	*S. parasitica*	434	Benin	Calavi, Cotonou	International Institute of Tropical Agriculture	6°25'N, 2° 20'E	*Eldana saccharina*	Maize
HT: A	*S. parasitica*	435	Benin	Calavi, Cotonou	International Institute of Tropical Agriculture	6°25'N, 2° 20'E	*Eldana saccharina*	Maize
HT: A	*S. parasitica*	437	Benin	Calavi, Cotonou	International Institute of Tropical Agriculture	6°25'N, 2° 20'E	*Eldana saccharina*	Maize
HT: A	*S. parasitica*	403	Kenya	Kilindini	Mombasa Island	4°02'S, 39°43'E	unknown	unknown
HT: B	*S. parasitica*	404	Senegal	Kaolack		14°15'N, 16°10'W	*Coniesta ignefusalis*	unknown
HT: C	*S. parasitica*	454	Kenya	Unknown			unknown	unknown
HT: D	*S. parasitica*	383	Zimbabwe	Harare	Agricultural Research Trust Farm	17°59'S, 30°81'E	*Busseola fusca*	Maize
HT: D	*S. parasitica*	429	Zimbabwe	Harare	Agricultural Research Trust Farm	17°59'S, 30°81'E	*Busseola fusca*	Maize
HT: D	*S. parasitica*	430	Zimbabwe	Harare	Agricultural Research Trust Farm	17°59'S, 30°81'E	*Busseola fusca*	Maize
HT: E	*S. parasitica*	382	Zimbabwe	Harare	Agricultural Research Trust Farm	17°59'S, 30°81'E	*Busseola fusca*	Maize
HT: E	*S. parasitica*	405	Zimbabwe	Harare	Agricultural Research Trust Farm	17°59'S, 30°81'E	*Busseola fusca*	Maize

United Kingdom), 1.5 mM MgCl$_2$, 200 μmol of each dNTP, 15 pmol of each PCR primer, 1 unit of Supertherm Gold Taq DNA Polymerase (JMR Holdings, United Kingdom), and approximately 250 ng of genomic DNA/RNA mix. The PCR product was purified using a QIAquick™ PCR Purification Kit (Qiagen), following the manufacturer's protocol. DNA sequencing was performed using an ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) under the following conditions: 32 cycles of (96 °C for 10 seconds, 50 °C for 30 seconds, 60 °C for 4 minutes), 4 °C hold. Sequencing products were cleaned using the manufacturer's Ethanol/Sodium Acetate /EDTA precipitation protocol. Sequences were visualized on an ABI 3100 Genetic Analyzer (Applied Biosystems).

Data Analyses

DNA sequence chromatograms were edited and checked for base-calling errors using the Staden package (Staden 1996). Sequences were aligned using ClustalX version 1.81 (Thompson *et al.* 1997) and then manually corrected using BioEdit version 7.0.1 (Hall 1999). Using TCS 1.21 (Clement *et al.* 2000) a haplotype network was reconstructed. MODELTEST version 3.7 (Posada & Crandall 1998) was used to select the substitution model that best described the data, using the Akaike Information Criterion (AIC). Phylogenetic analyses were performed under both maximum parsimony and maximum likelihood criteria using PAUP*4.0b10 (Swofford 2002). Maximum parsimony analysis comprised an exhaustive search and a 1000-replicate branch-and-bound bootstrap analysis. Maximum likelihood analysis used the model parameters selected by MODELTEST. Model parameters were estimated on the parsimony tree, and a branch-and-bound search then performed under the maximum likelihood criterion. Model parameters were then re-estimated, and fixed for subsequent maximum likelihood analysis. Another branch-and-bound search was performed, and a 1000-replicate branch-and-bound bootstrap analysis. Pairwise divergences between haplotypes and clades were calculated for both uncorrected and ML-corrected distances.

Results

A 454 bp DNA sequence alignment was obtained from 17 *Sturmiopsis* specimens (GenBank accession numbers DQ336397, DQ336399-DQ336413). Five haplotypes were found, and the network in shown on figure 1. The network consists of two unjoined groups, separated by a minimum of 15 substitutions. The first group contains haplotypes A and B, separated by one substitution. The second group contains three haplotypes: C and D and separated by one substitution, and E is separated from C by five hypothetical intermediate haplotypes.

A dataset comprising each of the five *S. parasitica* haplotypes and the single *S. inferens* haplotype was analysed by maximum parsimony. A single most parsimonious (MP) tree was recovered (fig. 2). The tree length was 51 steps, with a consistency index of 0.94 and a retention index of 0.89. The ingroup was divided into two main clades, one predominantly eastern in
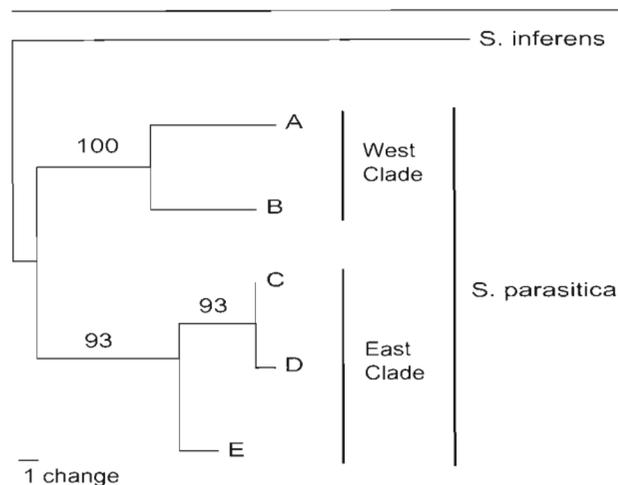
Figure 2
Single most parsimonious tree. Numbers above branches are percentage bootstrap support, shown only if >50%.

distribution and one predominantly western. The exceptions to this rule are specimens from Kenya, which occur in both clades (see table 1). The clades are strongly supported under parsimony analysis with bootstrap values of 93% and 100% for the eastern and western clades, respectively.

The model selected by MODELTEST was the Hasegawa, Kishino and Yano (1985) model with invariant sites (HKY+I). Model parameters were as follows: base frequencies, A = 0.28569 C = 0.16312 G = 0.14145 T = 0.40974; kappa = 4.5630361; proportion of invariable sites (I) = 0.858271. Subsequent ML analysis recovered the same tree as the parsimony analysis (−ln L = 850.87810). Bootstrap support under ML analysis was low, at only 64% and
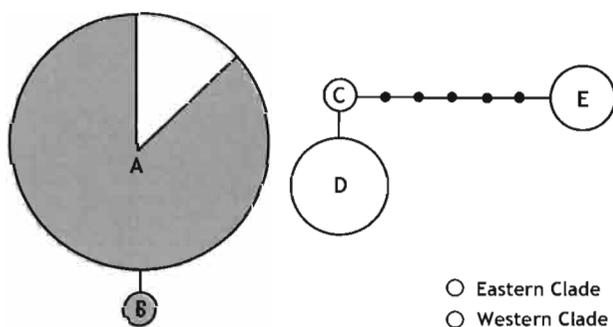
Figure 1
Haplotype network for *Sturmiopsis parasitica*. The surface of the circles are proportionate to the number of individuals having that haplotype. Single mutations are indicated by a line whereas hypothetical intermediate haplotypes are indicated by a circle. The network consists of two unjoined groups, separated by a minimum of 15 substitutions.

Table 2. DNA sequence divergences among *Sturmiopsis parasitica* haplotypes and *S. inferens*. Maximum likelihood-corrected percentage divergences shown above the diagonal, uncorrected percentage divergences shown below the diagonal.

	S. inferens	Haplotype A	Haplotype B	Haplotype C	Haplotype D	Haplotype E
S. inferens	–	12.321	12.640	13.978	13.300	12.932
Haplotype A		–	0.220	5.538	5.650	5.841
Haplotype B	7.504	0.222	–	4.980	5.077	5.283
Haplotype C	7.505	4.638	4.205	–	0.225	1.397
Haplotype D	7.489	4.626	4.198	0.222	–	1.662
Haplotype E	6.828	4.846	4.415	1.327	1.542	–

46% for the eastern and western clades, respectively.

DNA sequence divergence values are shown in Table 2. Uncorrected divergences between *S. inferens* and *S. parasitica* ranged from 6.8% to 7.5%, while uncorrected divergences between the eastern and western clades of *S. parasitica* ranged from 4.2% to 4.8%. Maximum uncorrected divergence values within the eastern and western clades were 1.5% and 0.2%, respectively.

Discussion

Phylogenetic analysis revealed the existence of two distinct clades within *S. parasitica*. The first clade predominantly consisted of specimens collected from *E. saccharina* in western Africa, whereas the second clade consisted of specimens collected from *B. fusca* in eastern and southern Africa. The exceptions were two specimens from Kenya, one belonging to the eastern clade and the other to the western clade.

There are two possible explanations for the results reported. First, we note that the phylogenetic separation of the Kenyan *S. parasitica* specimens is also observed in Kenyan *E. saccharina* (Assefa *et al.* 2006) and *B. fusca* (Sezonlin *et al.* 2006), where geographical features such as the Rift Valley play an important role

in dividing populations. Second, the western clade exclusively comprises specimens collected from the host insect *E. saccharina*, while eastern clade specimens were collected from *B. fusca*. Unfortunately we do not have host insect data for either of the Kenyan specimens, which would add an extra dimension to our data. The minimum uncorrected percentage DNA sequence divergence values between clades of *S. parasitica* is 4.2% which is high, based on previous studies of interspecific divergence values for the COI gene in Diptera: Cognato (2004) reported that the maximum uncorrected sequence divergences observed within dipteran species of the genera *Chysomya*, *Lucilia* and *Phytomyza* (see table 3) were 0.11%, 2.6% and 0.34% respectively, and the minimum among-species divergences were 3.5%, 0.7% and 6.7% respectively. Thus the maximum within-species sequence divergence obtained in our study (4.8%) is far more than that usually encountered within dipteran species. Evolutionary rate is know to vary among clades, therefore an argument based on divergence values alone cannot be conclusive, however the high divergence values observed with *S. parasitica* are at least highly suggestive of substantial genetic differentiation between clades. This genetic differentiation among parasitoid clades could be driven by specialization of the parasitoid on different host species or lineages. However, the data presented here is insufficient to distinguish between this hypothesis and the alternative, that the cause is geographic separation. In order to have a better understanding of these factors further work is needed, such as increased sample size, the use of additional genetic markers as well as samples from different populations, which parasitize different host species.

References

Assefa Y., Mitchell A., Conlong D.E. 2006. Phylogeography of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) *Annales de la Société Entomologique de France (N.S.)* **42(3-4):** 331-337.

Barraclough D.A. 2004. A taxonomic review of *Sturmiopsis* Townsend, 1916, an Old World genus of Tachinidae (Diptera) parasitising economically significant lepidopterous stem borers. *African Invertebrates* **45:** 7-19.

Chinwada P., Overholt W.A., Omwega C.O., Mueke J.M. 2004. Biology of *Sturmiopsis parasitica* (Diptera: Tachinidae) and suitability of three cereal stem borers (Lepidoptera: Crambidae, Noctuidae) for its development. *Annals of the Entomological Society of America* **97:** 153-160.

Table 3. Comparison of percentage uncorrected sequence divergences in the COI-COII region for three fly genera, summarized from Cognato (2004).

Genus	Within species divergence	Among species divergence
Chysomya	0.09–0.11	3.50–3.70
Lucilia	0.09–2.60	0.70–2.40
Phytomyza	0.22–0.34	6.70–7.60

Clement M., Posada D., Crandall K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1660.

Cognato A.I. 2004. Standard percent sequence difference for insects will not predict species boundaries. *Journal of Economic Entomology* 97: 1-17.

Conlong D.E. 1997. Biological control of *Eldana saccharina* Walker in South African sugarcane: Constraints identified from 15 years of research. *Insect Science and its Application* 17: 69-78.

Conlong D.E. 2000. Indigenous African parasitoids of *Eldana saccharina* (Lepidoptera: Pyralidae*). Proceedings of the South African Sugar Technologists Association* 74: 201-211.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. http://www.mbio.ncsu.edu/BioEdit/bioedit.html.

Martin A.J. 1999. *Effect of temperature on the development biology of* Sturmiopsis parasitica *Curran (Diptera: Tachinidae), a larval parasitoid of* Eldana saccharina *Walker (Lepidoptera: Pyralidae).* B. Sc. Honours Project, University of Natal, Durban, South Africa.

Nagarkatti S., Rao V.P. 1975. Biology of and rearing technique for *Sturmiopsis parasitica* (Curr.)(Diptera: Tachinidae), a parasite of graminaceous borers in Africa. *Bulletin of Entomological Research* 65: 165-170.

Posada D., Crandall K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Sezonlin M., Dupas S., LeRu B., LeGall P., Moyal P., Calatayud P. A., Giffard I., Faure N. & Silvain J. F. 2005. Phylogeography and population Genetics of the maize stalk borer Busseola fusca (Lepidoptera, Noctuidae) in sub-Saharan Africa. *Molecular Ecology* 15: 407-420.

Simon C., Frati F., Beckenbach A., Crespi B., Liu H. & Flook P. 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701.

Staden R. 1996. The Staden Sequence Analysis Package. *Molecular Biotechnology* 5: 231-241.

Swofford D.L. 2002. *PAUP* Phylogenetic analysis using parsimony (*And other methods)*, Version 4.0B10. Sinauer Associates, Sunderland, Massachusetts, USA.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. 1997. The ClustalX window interface: flexible strategies for multiple sequence alignment aided by quality and analysis tools. *Nucleic Acid Research* 24: 4876-4882.

Phylogeography of *Eldana saccharina* Walker (Lepidoptera: Pyralidae)

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Abstract. The pyralid moth *Eldana saccharina* Walker is an indigenous insect widely distributed throughout sub-Saharan Africa. Studies have shown that populations from West Africa have distinct behavioural differences compared to populations from East and southern Africa. In addition, the parasitoid guilds attacking populations in these different regions are markedly different. This marked geographical variation evoked a hypothesis of genetic differentiation. To evaluate this hypothesis a molecular analysis was conducted on populations of *E. saccharina* from throughout much of the species' range, using the cytochrome *c* oxidase subunit I (COI) region of the mitochondrial genome. A minimum spanning network and a maximum parsimony tree separated the 21 specimens into three distinct groups. Results revealed the presence of substantial genetic differentiation that is related to geographic variation.

Résumé. Phylogéographie d'*Eldana saccharina* Walker (Lepidoptera : Pyralidae). *Eldana saccharina* Walker est une pyrale indigène largement répandue en Afrique sub-saharienne. Des études antérieures ont montré que les populations d'Afrique de l'Ouest avaient un comportement différent en terme de préférence écologique par rapport aux populations d'Afrique de l'Est et du Sud. De plus, les parasitoïdes associés sont également différents selon l'origine géographique de cette pyrale. Ceci indique une possible différentiation génétique selon la distribution géographique d'*E. saccharina*. Cette hypothèse a été testée par l'analyse d'un fragment du gène mitochondrial codant pour la cytochrome c oxydase I (COI) sur les populations de pyrale. Le réseau d'haplotypes et l'analyse phylogénétique (maximum de parcimonie) ont permis de séparer les 21 spécimens analysés en trois groupes distincts. Les résultats révèlent l'existence d'une différentiation génétique substantielle en fonction de la localisation géographique de l'insecte.

Keywords: Phylogeography, *Eldana saccharina*, mitochondrial DNA, sugarcane, indigenous host plants.

Eldana saccharina Walker 1865 (Lepidoptera: Pyralidae) is indigenous to Africa and feeds on cultivated graminaceous crops as well as on several wild grasses and sedges (Conlong 1994). The insect is a key pest of sugarcane in western, eastern and southern Africa (Atkinson 1980; Conlong 2001). *E. saccharina* has been reported from maize and sorghum in southern Africa, but seldom causes significant damage in these crops in this region (Atkinson 1980). This is in contrast to West Africa, where it is a major crop spoiler of maize and sorghum (Kaufmann 1983; Sampson & Kumar 1985; Shanower *et al.* 1993). Previous studies have reported that the insect exhibits considerable behavioral variation, displaying differential responses to control agents (Carnegie *et al.* 1985; Conlong 2001; Mazodze

& Conlong 2003) and feeding on different host plants in various parts of Africa (Conlong 2001; Matama-Kauma *et al.* 2002; Atachi *et al.* 2005). The species' confusing behavioral patterns and diverse natural enemy guilds contrast with a lack of morphological diversity, making this species a prime candidate for molecular systematic analysis (Evans *et al.* 2000; Scheffer 2000; King *et al.* 2002). Due to the increase in the economic importance of the insect (Mazodze & Conlong 2003; Webster *et al.* 2005), there is an urgent need to control it, preferably through habitat management and biological control. Ascertaining the degree of relatedness among populations is a basic prerequisite for making informed decisions regarding natural enemy selection, management and correct interpretation of ecological investigations.

Mitochondrial DNA is inherited maternally, does not recombine, and has a relatively fast rate of evolution, making it useful for studies of phylogeography and intraspecific relationships (e.g. Sperling *et al.* 1999;
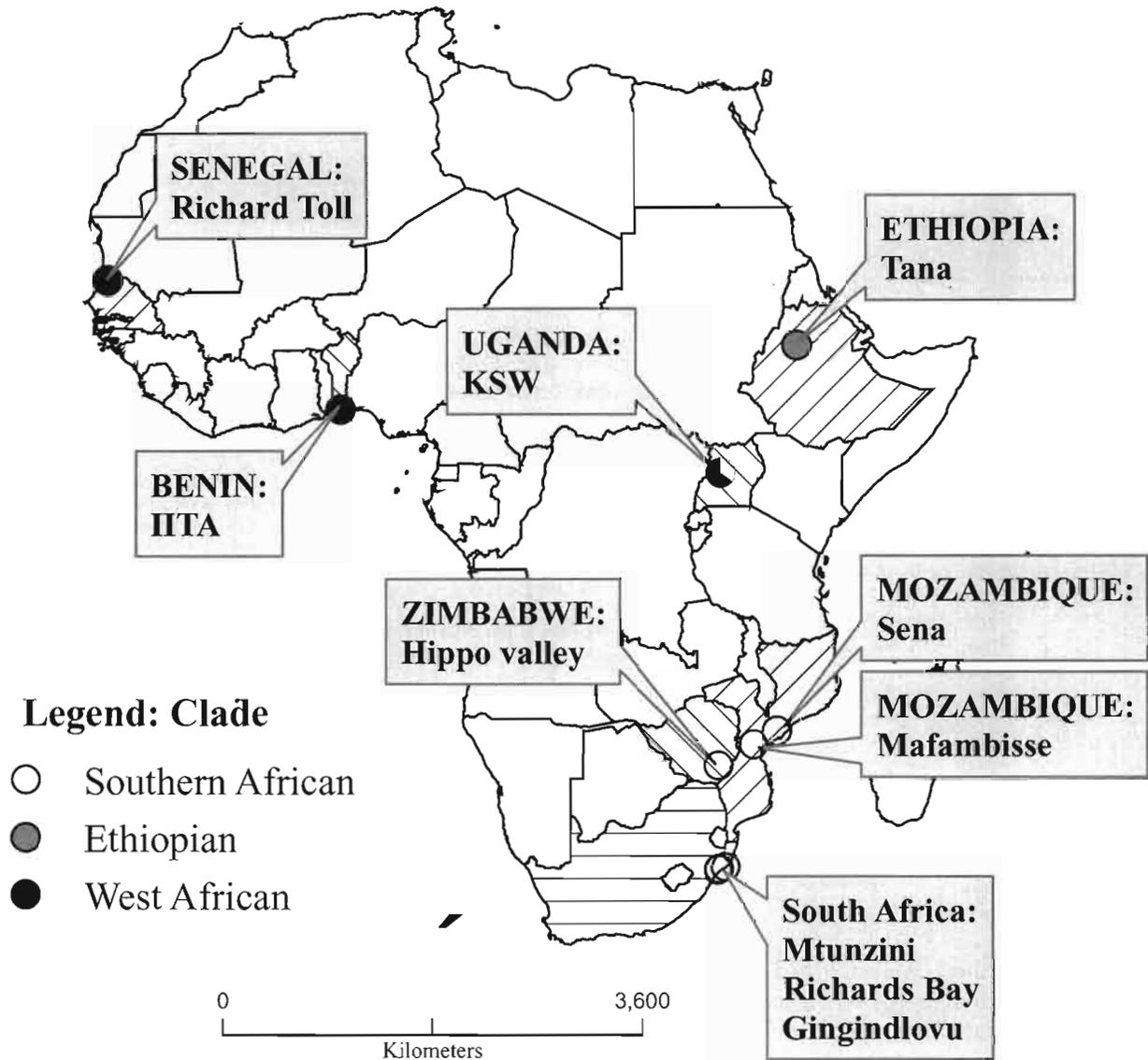
Figure 1
Map of Africa showing sampling localities in countries where *Eldana saccharina* was collected, and the distribution of haplotypes.

Scheffer 2000; Scheffer & Lewis 2001; Segraves & Pellmyr 2001; King *et al.* 2002; Simmons & Scheffer 2004). Mitochondrial DNA data also can reveal cryptic lineages representing distinct species or subspecies within geographically widespread and apparently morphologically homogeneous organisms (Scheffer 2000). King *et al.* (2002) provided the first evidence of the genetic structure of natural populations of *E. saccharina* and genetic variation among populations of the pest from different parts of Africa.

The present study is a re-examination and expansion of data presented in King *et al.* (2002)

where laboratory populations of *E. saccharina* from South Africa were compared with field populations from West and East Africa. In the current study, *E. saccharina* populations were collected in the field from three south African countries, namely South Africa, Mozambique and Zimbabwe. Moreover, this study expands the geographic area to include a north African population of *E. saccharina* from Ethiopia and stretches the western limit of the sampling up to Senegal. The addition of new taxa and populations can change relationships, sometimes drastically (Bucheli & Wenzel 2005). Thus, the aim of this study was to examine

Table 1. African locations where specimens of *Eldana saccharina* were collected.

DNA No	Accession No	Country	Location	Latitude, Longitude	Host	Haplotype
412	DQ486919	Ethiopia	Lake Tana	11°22'N, 31°39'E	*Cyperus papyrus*	G
413	DQ486918	Ethiopia	Lake Tana	11°22'N, 31°39'E	*Cyperus papyrus*	H
10	DQ486920	Benin	IITA Station	06°25'N, 02°20'E	Maize	J
11	DQ486926	Benin	IITA Station	06°25'N, 02°20'E	Maize	J
13	DQ486921	Benin	IITA Station	06°25'N, 02°20'E	Maize	K
220	DQ486924	Uganda	Kinyara Sugar Works	01°35'N, 31°36'E	Sugarcane	I
221	DQ486910	Uganda	Kinyara Sugar Works	01°35'N, 31°36'E	Sugarcane	F
276	DQ486925	Uganda	Kinyara Sugar Works	01°35'N, 31°36'E	Sugarcane	K
442	DQ486923	Senegal	Richard Toll	16°25'N, 15°42'W	Sugarcane	K
444	DQ486922	Senegal	Richard Toll	16°25'N, 15°42'W	Sugarcane	I
231	DQ486906	Zimbabwe	Cheredzi	21°0'S, 31°38'E	Sugarcane	A
298	DQ486913	Zimbabwe	Hippo valley estate	21°0'S, 31°38'E	*Cyperus digitatus*	F
297	DQ486908	Zimbabwe	Hippo valley estate	21°0'S, 31°38'E	*Cyperus digitatus*	A
299	DQ486917	Zimbabwe	Hippo valley estate	21°0'S, 31°38'E	*Typha latifolius*	E
233	DQ486911	Mozambique	Sena	18°17'S, 35°57'E	*Cyperus papyrus*	B
271	DQ486912	Mozambique	Mafambisse	19°20'S, 34°10'E	*Cyperus dives*	F
446	DQ486916	Mozambique	Sena	18°17'S, 35°57'E	*Cyperus papyrus*	F
448	DQ486914	Mozambique	Mafambisse	19°20'S, 34°10'E	*Cyperus dives*	F
80	DQ486907	South Africa	Richards Bay	28°48'S, 32°06'E	*Cyperus papyrus*	A
79	DQ486915	South Africa	Gingindhlovu West	29°02'S, 31°30'E	Sugarcane	D
300	DQ486909	South Africa	Mtunzini Farm	28°57'S, 31°39'E	*Cyperus dives*	C

whether genetic differentiation reported by King *et al.* (2002) operates on a much larger geographic scale, and to investigate the impact of geographic location on the genetic diversity of the species.

Materials and methods

Sample collection and DNA extraction

E. saccharina samples were collected from seven countries. The collection locality, date and the host plant from which each specimen was collected is indicated in tab. 1, along with the DNA extraction number of each sequence reported in this study. Of a total of 21 specimens of *E. saccharina* used in this study, only the three Benin specimens were included in the previous study by King *et al.* (2002). The additional 18 specimens were collected from sedges, maize and sugarcane from various localities in Ethiopia, Uganda, Senegal, Mozambique, Zimbabwe and South Africa (fig. 1). Genomic DNA was extracted from thoracic tissue using the Qiagen DNeasy™ Tissue Kit and stored at -20 °C. Voucher specimens (heads, abdomens and wings) are stored at the South African Sugarcane Research Institute (SASRI).

DNA amplification and sequencing

Polymerase Chain Reaction (PCR) amplifications were performed in a 50 µl volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 15 pmol of each PCR primer, 1 unit of SuperTherm Gold *Taq* DNA polymerase (JMR Holdings, United Kingdom) and 1 µl of genomic DNA. Primers used in the study were: Ron V (5'-GGAGCTCCAGATATAGCTTTCCC-3') and K525 (5'-ACTGTAAATATATGATGAGCTCA-3') (Loxdale & Lushai 1998) except for samples from Ethiopia, Senegal and

Mozambique (DNA No. 446 and 448 only), for which the primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer *et al.* 1994) was used instead of Ron V. PCR was performed using a Perkin Elmer GeneAmp PCR System 2400 (Applied Biosystems), under the following conditions: 94 °C for 11 min, 30 cycles of (94 °C for 30 s, 50-55 °C for 30 s, 72 °C for 30-90 s), 72 °C for 7 min, 4 °C hold. Amplified DNA was purified using the Qiagen QIAquick™ PCR purification kit following the manufacturer's protocol. DNA sequencing reactions were performed using the ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems), cleaned using Ethanol/EDTA precipitation with slight modification of the manufacturer's protocol, and sequences were visualized on an ABI 3100 Genetic Analyzer (Applied Biosystems).

Sequence analysis and phylogenetic reconstruction

Editing and assembling DNA sequence chromatograms was done using the Staden package (Staden 1996). Sequences were then aligned using ClustalX (Thompson *et al.* 1997) and manually corrected using BioEdit 5.0.9 sequence alignment editor (Hall 1999). *Galleria mellonella* (L. 1758) (Lepidoptera: Pyralidae) was used as outgroup because it belongs to the same subfamily (Galleriinae) as *E. saccharina*. A haplotype network was reconstructed using the statistical parsimony method of Templeton *et al.* (1992) in TCS 1.21 (Clement *et al.* 2000). Each haplotype was represented by a single sequence for phylogenetic analysis, which was performed by Maximum Parsimony (MP) in PAUP* v4.0b10 (Swofford 2002). The MP analyses used a branch and bound search, with tree reliability assessed by bootstrap analysis with 1000 replications. Only bootstrap values greater than 70% are reported.

Results

DNA sequence variation

Our analyses used the 509 bp region of the COI gene for which we obtained sequences for all individuals. The 21 COI sequences (including three from King *et al.* 2002, see above) were deposited in GenBank (accession numbers DQ486906 – DQ486926). Eleven haplotypes were identified of which six were unique (i.e. represented by single individuals). Six of the haplotypes (A-F) were from the southern African countries of Mozambique, South Africa and Zimbabwe (fig. 2). One specimen from Uganda was also included in the most common haplotype (F) of this 'south African clade'. The Ethiopian specimens had two separate haplotypes (G and H) that were distant from haplotypes from other parts of Africa. West African specimens from Senegal and Benin and the two remaining Ugandan specimens comprised three closely related haplotypes (I-K) (fig. 2).


● West African Population

○ southern African Population

◒ Ethiopian Population

◓ Ugandan Population
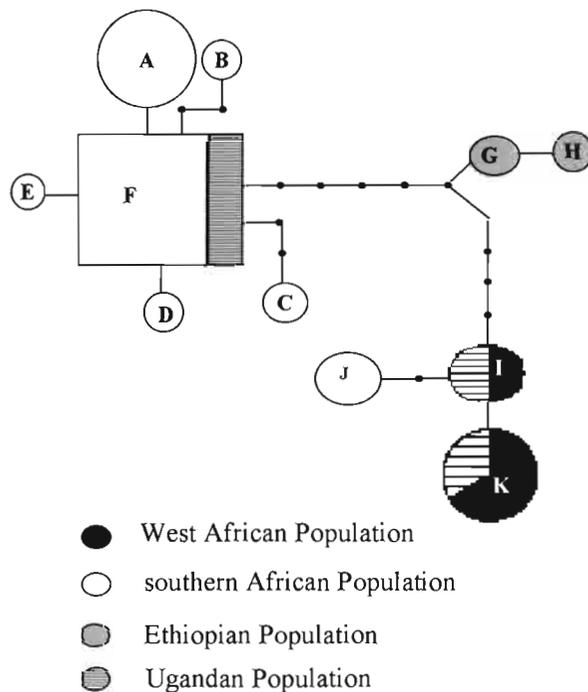
Figure 2
Haplotype network showing relationships between the 11 haplotypes. Each circle/square represents a single haplotype, with the size of the circles/square proportional to the number of individuals with that haplotype. Haplotypes that differed from each other by a single nucleotide mutation are connected by lines. Each small black circle represents one missing haplotype. Haplotype labels are the same as in tab. 1.

Phylogenetic analysis

The strict consensus of 2 MP trees (length = 64, retention index = 0.8889) is shown in fig. 3. Eighteen variable sites were found in the 509 bp fragment for which all specimens had data. Of these, 12 sites were parsimony-informative. Uncorrected pairwise sequence distances ranged from 0 to 2.4% (tab. 2). Three groups are apparent. The first group is a southern African group, including one sequence from Uganda, and shows within-group sequence divergence of up to 1.0%. This group has a moderate bootstrap support (71%; fig. 3). The second group is comprised of sequences from West Africa and Uganda. This group has a strong bootstrap support (87%; fig. 3). The third group is exclusively Ethiopian. This group also has a strong bootstrap support (92%; fig. 3). The phylogenetic tree is thus consistent with the haplotype network.

Discussion

Results of the phylogenetic analysis revealed that the Ethiopian specimens from west of the Rift Valley (Lake Tana) are almost identical to each other and different from both the west and southern African specimens. However, the number of specimens sampled from Ethiopia was very small (only two) and neighbouring countries such as Sudan and Kenya were not included in the study. This makes it difficult to generalize about relationship between the Ethiopian population of *Eldana saccharina* and populations from other parts of Africa. It is important to sample more specimens from Ethiopia and neighbouring countries to have a better understanding of the genetic diversity within the populations occurring in this region and their relationships with populations in other parts of Africa.

Similarly, West African specimens collected from a large geographic area were also very close to each other, indicating that *E. saccharina* in West Africa are part of an interbreeding population. The colonization of wide

Table 2. Uncorrected pairwise distances among sequences from various parts of Africa.

	West African Population	Ethiopian Population	Southern African Population
West African Population	**0.0-0.2**		
Ethiopian Population	1.2-1.6	**0.2**	
Southern African Population	1.6-2.4	1.2-2.0	**0.0-1.0**

areas by a single population of *E. saccharina* in West Africa could either be the result of even environmental selection (Hewitt 1999), as there are large areas of similar interlinking graminaceous vegetation types across West Africa, and very little geographical variation from the east to the west across West Africa. This lack of genetic variability could alternatively be the result of recent colonization of this area by *E. saccharina*. The degree of divergence observed in this group is within the limit of COI divergence observed in intraspecific comparison in other lepidopteran species (Cognato 2004).

The same is true for the southern African populations that are represented by 12 specimens from different localities in four countries, which show minimal variation between these populations from the south and eastern coastal part of Africa. The watercourses in western and especially southern Africa have not been disrupted as severely by earth movements as have those in East Africa since the Miocene (Beadle 1974a). It is thus likely that the climatic oscillations and vegetation diversity in these regions, caused by less drastic geographical variations, are relatively smaller to result in high genetic diversity.

In contrast to specimens from the rest of Africa, there was high genetic diversity among individuals from Uganda. Individuals from Kinyara Sugar Works in Uganda were distributed into both the western and southern clades detected in this study. The relatively high genetic diversity in Ugandan populations of *E. saccharina* as compared to populations from the rest of Africa could be associated with the impact of volcanic eruptions in Miocene and Pleistocene that significantly altered the hydrology of the region. Such massive volcanic eruptions and climatic change in the area could have modified the population structure of *E. saccharina*. Studies on vertebrate herbivores (Arctander *et al.* 1999; Alpers *et al.* 2004) and on *Busseola fusca* (Fuller 1901) (Lepidoptera: Noctuidae) (Sezonlin *et al.* 2006) have given enough evidence of the impact of these events on the distribution of different animal lineages in Africa.

These geological events and change in climate could affect the population structure of the pest in several ways. One of the possible explanations could be the formation of several hundreds of small crater lakes in the Western Rift on the Congo-Uganda border as a result of volcanic eruptions (Beadle 1974a). These lakes are mostly surrounded by swamps that are the natural habitat of indigenous host plants of *E. saccharina* (Atkinson 1980, Betbeder-Matibet 1981; Conlong 2001; Mazodze & Conlong 2003). The change in the water bodies affects the vegetation pattern of the
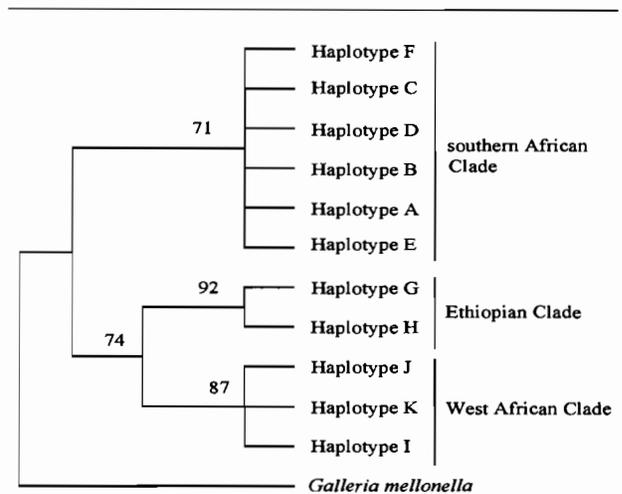
Figure 3
Strict consensus tree representing relationships of the eleven haplotypes of *Eldana saccharina*. The tree is rooted with *Galleria mellonella*. Numbers above internodes are bootstrap support values. Collection localities are indicated on a map of Africa (fig. 1). Haplotype names are indicated in fig. 2 and tab. 1.

area and can have an impact on the populations of *E. saccharina*. Some of the lakes in the Western Rift have no outlet and tend to be isolated from other water systems (Beadle 1974a) and result in discontinuous stand of wetland sedges and grasses. *Eldana saccharina* populations surviving in this region may have remained trapped and persisted by feeding on indigenous host plants in wetlands around these shallow lakes. As a consequence of prolonged isolation, the extant populations of *E. saccharina* situated close to each other could be highly divergent. However, the pattern of interactions between the lakes changed from time to time and some of these lakes became connected by rivers to other water systems (Beadle 1974a). It could be hypothesized that as wetland plants start to grow along the rivers, population at the western limits of the refugial range would expand into the areas of suitable territory in West Africa and those at the southern limits would expand to Southern Africa.

Another, more plausible hypothesis suggests that *E. saccharina* populations may have been isolated from each other because of the volcanic eruptions and climatic changes in Miocene and Pleistocene and survived in refugia in different parts of Africa. The populations of *E. saccharina* surviving in these were subjected to different selection pressures, which allowed genetic divergence from each other because of prolonged isolation and lack of gene flow between them. However, with time, the discrete populations may have expanded and colonized suitable vegetation within

their respective regions. They converged in Uganda because presently a network of swamps occupies about 6% of the total surface of Uganda (Wasawo 1964) and about sixty species of plants have been recorded in these swamps (Beadle 1974b). These diverse host plants available in a vast area of land are likely to be invaded by the populations from west and south through the continuous link of water bodies in the western Rift Valley. Thus, the increased genetic diversity observed in the Ugandan *E. saccharina* population could be a result of dispersal of the western and southern population into Uganda. This country, therefore, could be a "hybrid zone" where different clades of *E. saccharina* came in contact (Hewitt 1999).

More data are definitely needed to obtain a comprehensive understanding of the impact of geological events, climatic changes and geographic features on *E. saccharina*. For example, sampling populations of *E. saccharina* from geologically older wetlands such as the Sudd in Sudan, the Okavango Swamps in Botswana, and Lake Chad in Chad may provide more solid evidence on the relative ages of the *E. saccharina* populations, which would either substantiate or repudiate the recent colonization hypothesis.

These results are in agreement with previous reports from ecological studies that report variation in behavior, natural enemy complex and host plant preferences (Conlong 1994; 2000; 2001; Mazodze & Conlong 2003). In West Africa *E. saccharina* was reported to favor grasses to sedges in its natural habitat and is mainly a pest of maize (Betbeder-Matibet 1981). In southern Africa it prefers indigenous wetland sedges and sugarcane (Atkinson 1980). None of the natural enemies recorded in West Africa were found in southern Africa and vice versa (Conlong 2001). The genetic variation observed between the western and southern population of *E. saccharina* in this study also splits *E. saccharina* into groups that were reported from the ecological studies. Therefore, it is highly likely that the differences in behavior, host plant preference and natural enemy complex observed among populations of *E. saccharina* in different regions of Africa in previous studies could be result of genetic differentiation that the insect has undergone. The further separation of Ugandan specimens into west and southern groups strengthens this view. In a study conducted by Mazodze and Conlong (2003) in Zimbabwe and South Africa, *E. saccharina* was frequently found in the lower third of sugarcane stalks of older plants. However, in Kenya and Uganda the insect was found in the top third and middle of the stalk (Conlong 2001). Moreover, Conlong (2001) reported that West African and southern African parasitoid populations merged in Uganda. In this part

of the continent, *E. saccharina* is known to be a pest of both maize and sugarcane, which is uncommon in West and southern Africa (Girling 1972; Conlong & Mugalula 2001; Matama-Kauma *et al.* 2002). Hence, it is likely that Uganda is a place where the different populations of *E. saccharina* are found together.

The Ethiopian population of *E. saccharina* shares behaviours both with the southern and the western populations. In surveys conducted in wetlands and sugarcane fields of Ethiopia, *E. saccharina* was recovered only from indigenous wetland sedges but the natural enemy guilds attacking this pest in the country were similar to those reported from West Africa (Conlong *in lit.*). Results of genetic analysis in this study suggest that the Ethiopian population of *E. saccharina* is a separate group. It will be important to include specimens from countries between Ethiopia and West Africa to have a better understanding of the relationship between the Ethiopian and the West African populations of *E. saccharina*.

However, with current data from only seven African countries and six host plants, no associations between host plant and genetic variability could be established. The number of individuals included from each host plant from different countries was not sufficient to infer the impact of host plants.

Conclusion

Results of the current study show the existence of considerable genetic diversity between populations of *E. saccharina* from West Africa, Ethiopia and southern Africa. In contrast, genetic diversity within these three populations was very low. Specimens representing two of these three clades were found in Uganda. It is proposed that extreme geographic perturbations that occurred in East Africa during the Miocene and then habitat linkages re-establishing during the Pleistocene were the main drivers of this genetic diversity.

References

Alpers D.L., Van Vuuren B.J., Arctander P., Robinson T.J. 2004. Population genetics of the roan antelope (*Hippotragus equinus*) with suggestions for conservation. *Molecular Ecology* **13:** 1771–1784.

Arctander P., Johansen C., Coutellec-Vreto M. 1999. Phylogeography of three closely related African bovids (tribe *Alcelaphini*). *Molecular Biology and Evolution* **16:** 1724–1739.

Atachi P., Sekloka E.T., Schulthess F. 2005. Study on some bioecological aspects of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) on *Zea mays* L. and alternative host plants. *Journal of Applied Entomology* **129** (8): 447-455.

Atkinson P.R. 1980. On the biology, distribution and natural host-plants of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Journal of Entomological Society of Southern Africa* **43**: 171-194.

Beadle L.C. 1974a. Geological and climatic history, present climate, p. 17-33 *in*: **Beadle L. C. (ed.),** *The inland waters of tropical Africa: An introduction to tropical lumnology.* Longman Group Ltd, London.

Beadle L.C. 1974b. Tropical swamps: Adaptation to scarcity of oxygen, p. 241-258 *in*: **Beadle L. C. (ed.),** *The inland waters of tropical Africa: An introduction to tropical lumnology.* Longman Group Ltd, London.

Betbeder-Matibet M. 1981. *Eldana saccharina* Walker. Borer of the stalk of sugarcane in Africa. *L'Agronomie Tropicale* **36**: 279-293.

Bucheli S.R., Wenzel J. 2005. Gelechioidea (Insecta: Lepidoptera) systematics: A reexamination using combined morphology and mitochondrial DNA data. *Molecular Phylogenetics and Evolution* **35**: 380-394.

Carnegie A.J.M., Conlong D.E., Graham D.Y. 1985. Recent introduction of parasitoids against *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Proceedings of the South African Sugar Technologists' Association* **40**: 160-163.

Clement M., Posada D., Crandall K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1659.

Cognato A.I. 2004. Standard percent sequence difference for insects will not predict species boundaries. *Journal of Economic Entomology* **97**: 1-17.

Conlong D.E. 1994. A review and perspectives for the biological control of the African sugarcane stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Agriculture, Ecosystem and Environment* **48**: 9-17.

Conlong D.E. 2000. Indigenous African parasitoids of *Eldana saccharina* (Lepidoptera: Pyralidae). *Proceedings of the South African Sugar Technologists' Association* **74**: 201-211.

Conlong D.E. 2001. Biological control of indigenous African stemborers: what do we know? *Insect Science and its Application* **21**: 267-274.

Conlong D.E., Mugalula A. 2001. *Eldana saccharina* (Lep: Pyralidae) and its parasitoids at Kinyara sugar works, Uganda. *Proceedings of the South African Sugar Technologists' Association* **75**: 183-185.

Evans J. D., Pettis J. S., Shimanuki H. 2000. Mitochondrial DNA relationships in an emergent pest of honey bees *Aethina tumida* (Coleoptera: Nitidulidae) from the United States and Africa. *Annals of the Entomological Society of America* **93**: 415–420.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294-297.

Girling D.J. 1972. *Eldana saccharina* Wlk. (Lepidoptera: Pyralidae), a pest of sugarcane in East Africa. *Proceedings of the International Society of Sugarcane Technologists* **14**: 429-434.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *http://www.mbio.ncsu.edu/BioEdit/bioedit.html*

Hewitt G.M. 1999. Post-glacial re-colonization of European biotas. *Biological Journal of the Linnean Society* **68**: 87-112.

Kaufmann T. 1983. Behavioural biology, feeding habits, and ecology of three species of maize stem-borers: *Eldana saccharina* (Lepidoptera: Pyralidae), *Sesamia calamistis* and *Busseola fusca* (Noctuidae) in Ibadan, Nigeria, West Africa. *Journal of the Georgian Entomology Society* **18**: 259-272.

King H., Conlong D.E., Mitchell A. 2002. Genetic differentiation in *Eldana saccharina* (Lepidoptera: Pyralidae): evidence from the mitochondrial cytochrome oxidase I and II genes. *Proceedings of the South African Sugar Technologists' Association* **76**: 321-328.

Loxdale H.D., Lushai G. 1998. Molecular markers in entomology. *Bulletin of Entomological Research* **88**: 577-600.

Matama-Kauma T., Kyamanywa S., Ogwang J.A., Omwega C.O., Willson H.R. 2002. Cereal stemborer species complex and establishment of *Cotesia flavipes* Cameron in eastern Uganda. *Insect Science and its Application* **22**: 317-325.

Mazodze R., Conlong D.E. 2003. *Eldana saccharina* (Lepidoptera: Pyralidae) in sugarcane (*Saccharum* hybrids), sedge (*Cyperus digitatus*) and Bulrush (*Typha latifolia*) in south-eastern Zimbabwe. *Proceedings of The South African Sugarcane Technologists' Association* **77**: 266-274.

Sampson M.A., Kumar R. 1985. Life history, development and behaviour of *Eldana saccharina* Walker on sugarcane in southern Ghana. *Insect Science and its Application* **6**: 135-143.

Scheffer S.J. 2000. Molecular evidence of cryptic species within *Lyriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology* **93**: 1146–1151.

Scheffer S.J., Lewis M.L. 2001. Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Annals of the Entomological Society of America* **94**: 648–653.

Segraves K.A., Pellmyr O. 2001. Phylogeography of the yucca moth *Tegeticula maculata*: the role of historical biogeography in reconciling high genetic structure with limited speciation. *Molecular Ecology* **10**: 1247–1253.

Sezonlin M., Dupas S., Le Rü B., Le Gall P., Moyal P., Calatayud P-A., Giffard I., Faure N., Silvain J.F. 2006. Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa. *Molecular Ecology* **15**: 407-420.

Shanower T.G., Schulthess F., Bosque-Perez N.A. 1993. Effect of larval diet on the growth and development of *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Insect Science and its Application* **5/6**: 681-685.

Simmons R.B., Scheffer S.J. 2004. Evidence of cryptic species within the pest *Copitarsia decolora* (Guene´e) (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **97**: 675-680.

Sperling F.A.H., Raske A.G., Otvos I.S. 1999. Mitochondrial DNA sequence variation among populations and host races of *Lambdina fiscellaria* (Gn.) (Lepidoptera: Geometridae). *Insect Molecular Biology* **8**: 97-106.

Staden R. 1996. The Staden Sequence Analysis Package. *Molecular Biotechnology* **5**: 233-241.

Swofford D.L. 2002. *PAUP* Phylogenetic analysis using parsimony (*And other methods)*, Version 4.0B10. Sinauer Associates, Sunderland, Massachusetts, USA.

Templeton A.R., Crandall K.A., Sing C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. 3. Cladogram estimation. *Genetics* **132**: 619–633.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876-4882.

Wasawo D.P.S. 1964. Some problems of Uganda swamps, p 196-204 *in*: **Morges (ed.),** *The ecology of man in the tropical environment.* International Union for Conservation of Nature and Natural Resources (IUCN) Publications New Series No. 4. Switzerland.

Webster T.M., Maher G.W., Conlong D.E. 2005. An integrated pest management system for *Eldana saccharina* in the midlands north region of KwaZulu-Natal. *Proceedings of the South African Sugar Technologists' Association* **79**: 347-358.

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Phylogeographic pattern and regional evolutionary history of the maize stalk borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in sub-Saharan Africa

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Abstract. *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is one of the major cereal pests in sub-Saharan Africa. Previous phylogeographic investigations on samples collected in Kenya, Cameroon and West-Africa showed the presence of three main clades (*W, KI, KII*) originated from populations isolated in West and East Africa around one million years ago. Demographic and phylogenetic analyses suggested that this event was followed by local demographic expansion and isolation by distance. These hypotheses were tested by a more comprehensive sampling across *B. fusca*'s geographic range in Africa. Comparisons of sequences of partial mitochondrial DNA gene (cytochrome *b*) from 489 individuals of 98 localities in southern, central, eastern and western African countries confirmed the presence of the three main clades. Phylogenetic, F-statistics, demographic parameters and nested clade phylogeographic analyses confirmed that the clades experienced geographic and demographic expansion with isolation by distance after their isolation in three refuge areas. The geographic range of clade *KII*, already known from East to Central sub-Saharan Africa was extended to Southern Africa. Mismatch distribution analysis and the negative values of Tajima's D index are consistent with a demographic expansion hypothesis for these three clades. Significant genetic differentiations were revealed at various hierarchical levels by analysis of molecular variance (AMOVA). Hypotheses about the geographic origin of the three main clades are detailed.

Résumé. Scénario phylogéographique et histoire évolutive régionale du foreur de graminées *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae) en Afrique sub-saharienne. *Busseola fusca* (Fuller) (Lépidoptère : Noctuidae) est l'un des ravageurs majeurs des cultures céréalières en Afrique Subsaharienne. Une première étude phylogéographique portant sur des individus échantillonnés au Kenya, au Cameroun et en Afrique de l'Ouest a montré l'existence de trois clades principaux (*W, KI, KII*) issus de populations isolées à l'Ouest et à l'Est de l'Afrique il y a environ un million d'années. Les analyses démographiques et phylogénétiques indiquent que cet événement a été suivi d'une expansion démographique locale avec des phénomènes d'isolement par la distance. Ces hypothèses ont été testées à plus grande échelle grâce à un échantillonnage des populations de *B. fusca* couvrant désormais la majeure partie de son aire de distribution. Le séquençage d'un fragment du gène mitochondrial codant pour le cytochrome *b* chez 489 individus provenant de 98 localités des pays sud, centre, est et ouest africains confirme l'existence des trois clades observés précédemment. Les résultats des analyses phylogénétiques, les paramètres démographiques, les statistiques de Wright ainsi que les analyses des clades emboîtés confirment que ces trois populations, après avoir été isolées dans des aires refuges différentes, ont connu une expansion démographique et géographique avec un isolement par la distance. La distribution géographique du clade *KII*, connue de l'Afrique l'Est à l'Afrique centrale, s'étend jusqu'en Afrique Australe. L'analyse de 'mismatch distribution' et les valeurs négatives de l'indice D de Tajima sont bien en accord avec l'hypothèse d'une expansion démographique de ces trois clades. Des différenciations génétiques significatives ont été révélées aux différents niveaux hiérarchiques par l'analyse moléculaire de la variance (AMOVA). Les hypothèses sur l'origine géographique des trois clades sont précisées.

Keywords: Stem borer, population genetics, cytochrome *b*, Pleistocene, Africa.

The most important cereal crops in sub-Saharan Africa are maize, millet, rice and sorghum. Most of these cereal crops serve as host plants for many stem borer insects among which *Busseola fusca* (Fuller 1901) (Lepidoptera: Noctuidae) is one of the most economically important pests (Polaszek & Khan 1998). *B. fusca* is an endemic species in sub-Saharan Africa with wide geographical distribution (Ajayi 1998; Moyal 1998; Ndemah *et al.* 2001; Haile & Hofsvang 2001; Kfir *et al.* 2002). It is a major pest of sorghum (*Sorghum bicolor* [L.] Moench) and maize (*Zea mays* L.) (Poaceae). The domestication of sorghum probably began some 5,000 years ago in North-East Africa (Dogett 1988; Murty & Renard 2001) whereas maize was introduced more recently, at the end of the 16th Century (Madeira Santos & Ferraz Torrao 1998; Chastanet 1998). *Busseola fusca* varies in its ecological preference across its geographical range. It is more adapted to lowland in West Africa than in East and Southern Africa (Kfir *et al.* 2002). Previous study on the genetic structure of *B. fusca* shows that this ecological preference is associated with major differences in partial DNA sequence of the cytochrome *b* mitochondrial gene (Sezonlin *et al.* 2006). According to that study, *B. fusca* populations are differentiated into three major clades of mitochondrial haplotypes, one located in West African region (*W*), one restricted to East Africa (*KI*) and one found in Central and East Africa (*KII*). The origin of these clades is likely related to Pleistocene climatic events. Partial geographic overlap was observed only between clades *KI* and *KII*. Biogeographic barriers likely corresponding to *B. fusca* ancient history on wild Poaceae have been shown to be the major factors of differentiation of this species. These barriers, namely the Cameroon Volcanic Line (CVL) region between Central and West Africa and the Rift Valley in East Africa, appear to be similar to those that shaped geographic differentiation of phytophagous mammals and rodents (Sezonlin *et al.* 2006).

However, no signature of sorghum domestication and maize introduction has been detected yet on the genetic structure of *B. fusca*, despite the expected important demographic consequences of this switch to cultivated plants.

The regional evolutionary history and the centres of origin of the mitochondrial clades remain unknown. To answer these questions and to more accurately estimate geographical distribution of each clade, the sampling of *B. fusca* was completed to include most of its geographic range from Western, Eastern to Southern Africa. Samples from Eritrea, Ethiopia, Malawi, Mozambique, Rwanda, Republic of South Africa, Uganda, Zambia and Zimbabwe were added.

Partial sequences of the gene coding for cytochrome *b*, informative at the intrageneric level in Lepidoptera (Simmons & Weller 2001; Sezonlin *et al.* 2006) have been used. A sequential approach combining several phylogeographic and evolutionary methods (Bernatchez 2001) was used to analyse the molecular data and infer the demographic history of *B. fusca* populations in more details. Such analyses in sequential approach that start from phylogeny to evolutionary history via demography and genetic structure allow us to move from testing deeper phylogenetic splits to inferring recent patterns of population structure. This also may highlight the centres of origin of *B. fusca* populations and may elucidate the regional evolutionary history that has produced this genetic structure.

Material and methods

Moth sampling

The sampling of *Busseola fusca* individuals was carried out between 2001 and 2004. *B. fusca* individuals were sampled in West Africa (19 localities from Benin, Togo, Ghana, Mali and Burkina-Faso), in Central Africa (3 localities from Cameroon), in East Africa (55 localities from Kenya, Uganda, Rwanda, Tanzania, Ethiopia and Eritrea) and in Southern Africa (21 localities from Malawi, Mozambique, Zambia, Zimbabwe and Republic of South Africa) (figs.1a, b, c, d).

Moths rearing and conservation

Larvae and pupae collected were brought to the laboratories (IITA - Cotonou for Central and West Africa and ICIPE - Nairobi for Southern and Eastern Africa) to be reared to adulthood on semi-natural medium made of fresh stems of maize and cultivated sorghum (IITA) and artificial medium (ICIPE). The rearing of larvae allows the morphological identification of *B. fusca* moths among other stem borers species. The moths were killed just after emergence and preserved in absolute ethanol before DNA extraction.

Molecular analysis

Total DNA was extracted from insect thoraxes, using the DNeasy tissue kit (Qiagen GmbH, Germany). The number of individuals of *B. fusca* analyzed for each locality ranged from one to 13. The molecular marker used is a fragment of the gene coding for the cytochrome *b* for which approximately 1000 bp were amplified by PCR. The same primers and PCR protocol as described by Sezonlin *et al.* (2006) were used for all samples. Amplified PCR products were purified with the Quick protocol (Promega Wizard SV Gel and PCR Clean-Up System) and directly sequenced on an automated sequencer ABI prism 377 using the amplification primers in both directions. The consensus sequences obtained were aligned manually using MacClade 4.06 (Maddison & Maddison 2002).

Haplotype phylogeny

Phylogenetic relationships were estimated by means of maximum parsimony (MP) and Neighbour-Joining (NJ) using Maximum-Likelihood distances with PAUP* 4b10 (Swofford
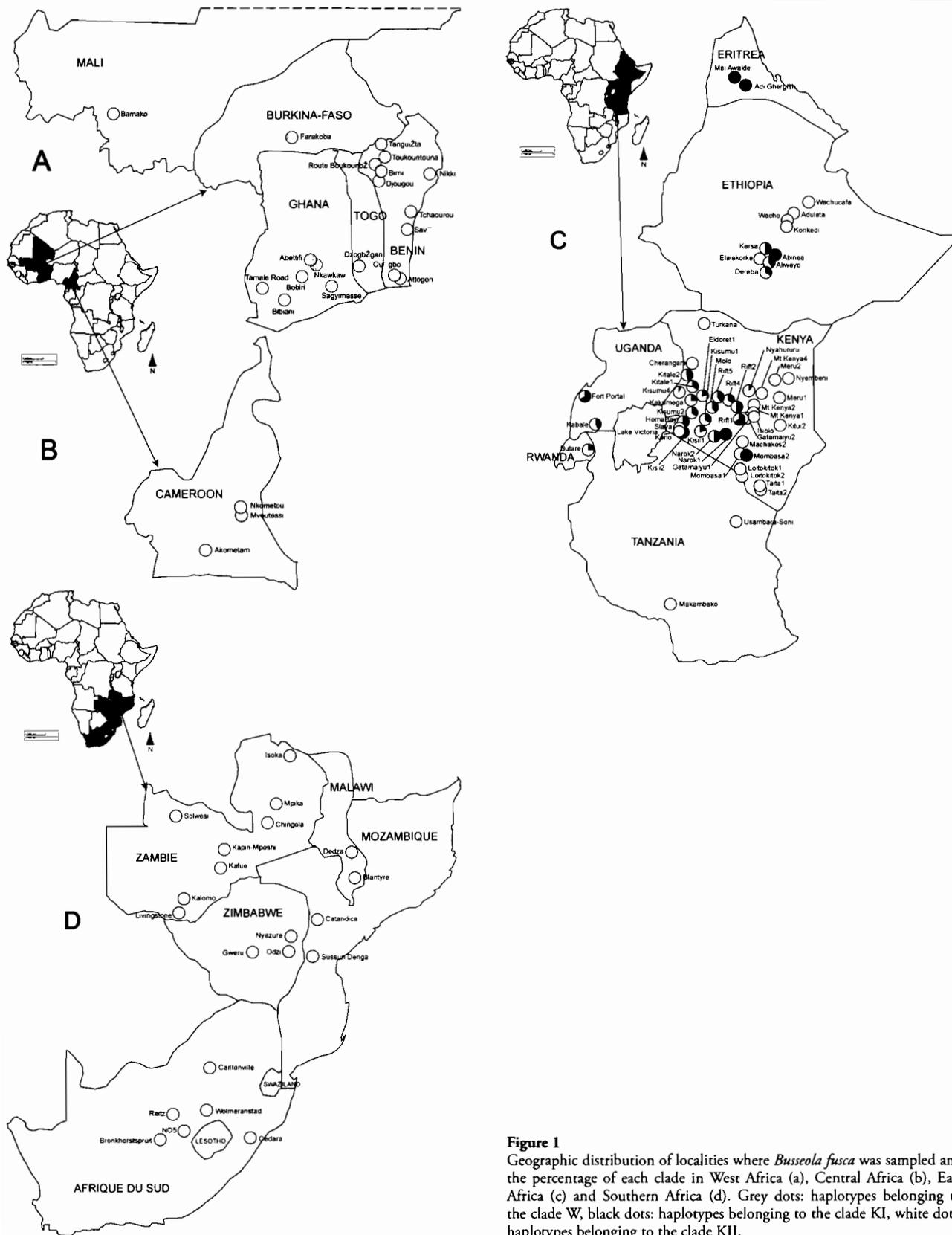
Figure 1
Geographic distribution of localities where *Busseola fusca* was sampled and the percentage of each clade in West Africa (a), Central Africa (b), East Africa (c) and Southern Africa (d). Grey dots: haplotypes belonging to the clade W, black dots: haplotypes belonging to the clade KI, white dots: haplotypes belonging to the clade KII.

2002). MP analyses were performed using a heuristic search strategy starting with stepwise addition trees replicated 10 times, using a random input order of sequences to get the initial tree for each replicate. Robustness of MP topologies was assessed by bootstrap with 100 replicates (full heuristic search) of 10 random stepwise addition replicates each, for all analyses.

Although the systematics of African lepidopteran stem borers is still rather confused (Holloway 1998), recent studies showed that *Busseola phaia* Bowden 1956 (Lepidoptera: Noctuidae), collected from various regions of East Africa, is the sister species of *B. fusca* (Moyal, *pers. com.*). Therefore, *B. phaia* was chosen as outgroup.

MODELTEST version 3.07 (Posada & Crandall 1998) was used to select the substitution model(s) that best describe the data. This software performs a hierarchical test of likelihood fits under 56 different models of character variation.

Diversity indices and demographic history of *Busseola fusca* clades

Haplotypic (h) and nucleotide (π) diversity values and all demographic parameters were performed with ARLEQUIN 2.000 software (Schneider *et al.* 2000) for groups of haplotypes in order to estimate their level of polymorphism and to localize the centre of origin of the different clades. These diversity indices are useful to examine the demographic history of a lineage (Grant & Bowen 1998) because their value does not depend on the length of the DNA fragment, nor on the sample size (Nei & Li 1979; Nei 1987). The centre of origin of each *B. fusca* clade was established by comparing the genetic diversity for different groups defined within this main population. In this case, populations were grouped according to geography in Central, South and East Africa and phytogeographic zones (White 1983) in West Africa. In West Africa three phytogeographic zones concerned our study, forest (drier types) region, forest and secondary grassland region and savannah region whereas in the rest of Africa, four geographic zones were retained, namely East – North, East – South, Austral and Cameroon. Centres of origin would have higher haplotype and nucleotide diversity than more recently founded populations (Althoff & Pellmyr 2002). This analysis could allow us to track the recent geographical expansions of these populations. The distribution of pairwise differences between individual sequences was analyzed by means of mismatch distribution analysis (Slatkin & Hudson 1991; Schneider & Excoffier 1999). A unimodal distribution would be expected for populations in expansion or for populations that have undergone a recent bottleneck, and a multimodal distribution for populations at demographic equilibrium (Slatkin & Hudson 1991). The raggedness index of the observed distribution (r) representing the modality of the distribution, and the sum of square deviation from the mismatch expected from a model of sudden population expansion (SSD) were calculated. Since the nucleotide substitution models selected by hierarchical likelihood ratio tests (hLRTs) (HKY + I + G) and Akaike information criterion (AIC) (K81uf + I + G) were not available in the ARLEQUIN 2.000 software, the r and SSD indices were calculated by using pairwise differences. The significance of these statistics was tested as implemented in ARLEQUIN. Finally, Tajima's D index was calculated with ARLEQUIN. This index can provide information about demographic history with demographic expansion leading to negative values, and subdivided populations leading to positive values (Tajima 1989a, b).

Genetic structure of *Busseola fusca* populations

In order to test for genetic differentiation, hierarchical levels of genetic divergence between various groups were calculated with the fixation index Φ_{ST} (Excoffier *et al.* 1992), an estimator that includes information on haplotype frequency and molecular distance. The significance of Φ_{ST} for population comparisons was assessed using 1000 permutations. The Φ_{ST} values and permutations were computed in ARLEQUIN 2.000 (Schneider *et al.* 2000).

Nested clade phylogeographic analysis (NCPA)

NCPA was performed as described by Templeton (1998, 2004). The genealogic relationships are represented through a haplotype parsimony network to define a series of nested clades. The probabilities of haplotype connections were calculated according to coalescent theory using TCS1.21 software (Clement *et al.* 2000) and the network with probabilities above the parsimony threshold (0.95) was selected. The hypothesis of random geographic distributions is tested through permutation tests for each clade and subclade components. These statistical analyses of geographical distances within and between clades were carried out with GeoDis 2.1 (Posada *et al.* 2000). GPS coordinates of all sampling localities were used. The geographical distances between centres of distributions of clades were tested for significance in permutation tests, within clade (*Dc*, the average distance of individuals from the clade's geographical centre), with nested clade centre (*Dn*, the average distance of individuals from the geographical center of all members of the nested clade) or between interior and tip at each level (*(I-T)Dc*, the average distance between interior and tip clades within a given clade and *(I-T)Dn*, the average distance between interior and tip clades in the nested clade). Significant geographic patterns were interpreted in terms of population history, using the latest inference key from Templeton (2004) from http://darwin.uvigo.es.

Results

Phylogenetic reconstruction

A fragment of 965 bp encoding cytochrome *b* was sequenced from 489 individuals of *Busseola fusca* across its geographic range from Western, Central, Southern and Eastern Africa. We observed 108 different haplotypes (GenBank accession numbers AY769536 to AY769605 and DQ284857 to DQ284895). The haplotypes with GenBank accession number AY769536 to AY769605 were re-used whereas those with accession number DQ284857 to DQ284895 were news. 123 nucleotide sites were variable (12.75%) and 58 were informative in parsimony analysis (6.01%). Parsimony analysis generated 438 equiparsimonious trees (length = 342, CI = 0.371, RI = 0.808). All trees were divided into the same three clades: a clade grouping sequences from the West African region only (*W*), a Kenya I clade (*KI*) and a Kenya II clade (*KII*), which also contained sequences from Cameroon and southern African countries (fig. 2). Discrepancies between these 438
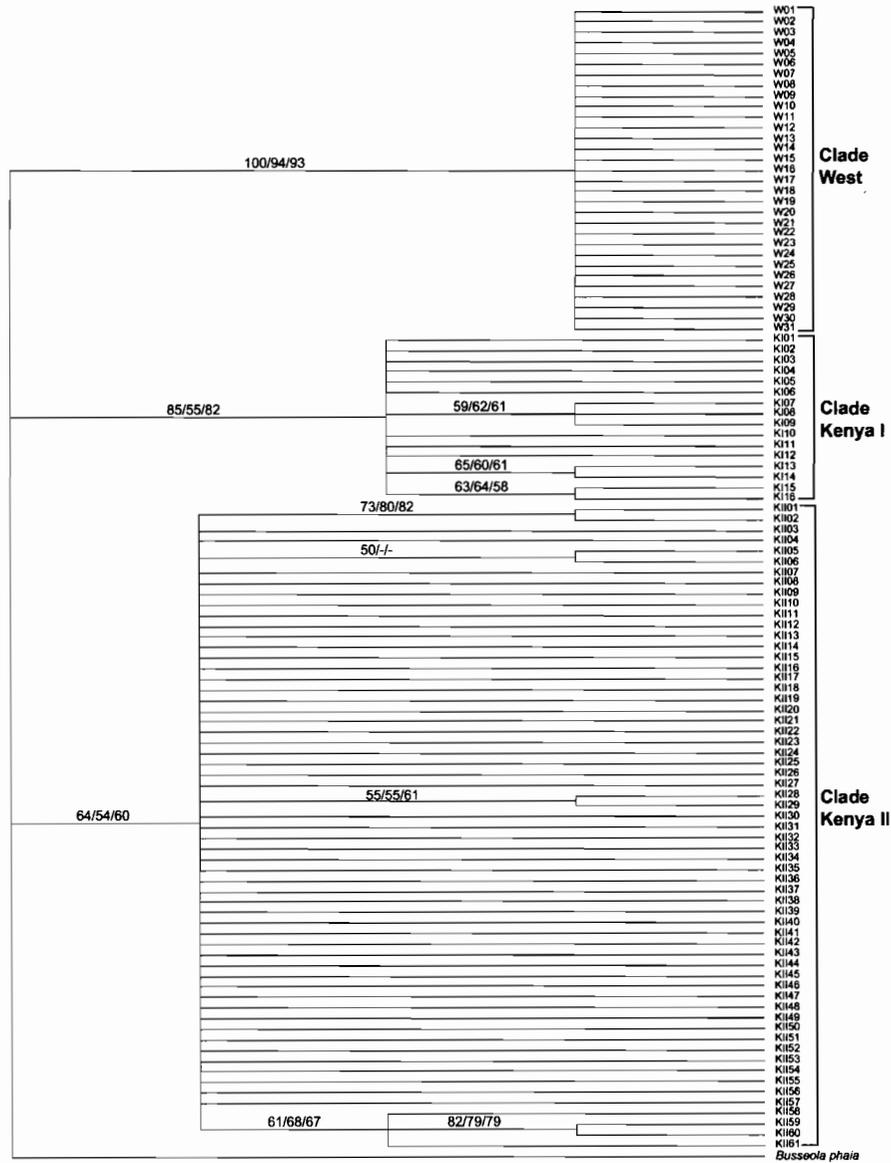
Figure 2
Majority consensus of the most parsimonious trees. Bootstrap support of >50% in both MP (first number), NJ with hLRT (second number), and NJ with AIC (third number) searches in 1000 replicates are given for the relevant nodes. *Busseola phaia* was used as the outgroup taxon.

equiparsimonious topologies concerned only the apical nodes. It was therefore possible to construct a majority rule consensus of the most parsimonious trees (fig. 2).

The model selected by the Akaike information criterion of the maximum likelihood (ML) was the K81uf + I + G (-LnL = 2479.6731) (Kimura 1981). The parameters inferred from this substitution model were: A =0.337, C= 0.140, G = 0.106, T = 0.417;

[AC substitution rate] = 1.000, [AG] = 22.5462, [AT] = 0.3998, [CG] = 0.3998, [AT] = 22.5462, [GT] = 1.000 with a certain proportion of invariable sites (I = 0.6918) and heterogeneous rate of substitution following a gamma distribution with alpha shape α = 0.7750. According to the hierarchical likelihood ratio tests (hLRT), the HKY + I + G model of evolution (-LnL =2480.8875) (Hasegawa *et al.* 1985; Yang 1993;
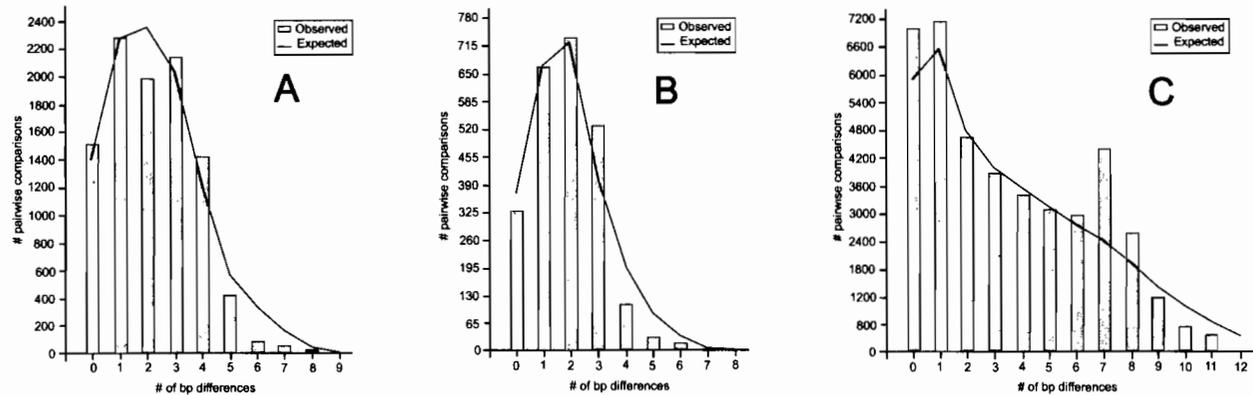
Figure 3
Mismatch distribution analysis showing histogram of observed and expected mismatch frequencies (A: West Africa population; B: Kenya I population; C: Kenya II population).

Gu *et al.* 1995) was selected. The parameters estimations were I = 0.6904 and α = 0.7714. Neighbour-Joining analyses of the ML distances obtained using the parameter estimates derived from each substitution model were performed. Bootstrap values for each NJ analysis were obtained from 1000 replications. Topology of NJ tree obtained using ML distances was similar to the one derived from MP analyses (fig. 2).

As already pointed out by Sezonlin *et al.* (2006), the three conspicuous clades of individuals and haplotypes are supported by bootstrap values exceeding 50% in both MP an NJ analyses. The smallest clade *KI* comprised 16 haplotypes and 70 individuals, all of which came from East Africa. The clade *KII* comprised 61 haplotypes and 280 individuals and had the largest

Table 1. Estimates of haplotype and nucleotide diversity for different population groupings of *B. fusca*. Forest region populations have the highest diversity for clade *W*, East-North region populations have the highest diversity for clade *KI*, East-South region populations have the highest diversity for clade *KII*.

| Clade	Region	Haplotype diversity	Nucleotide diversity (%)
W	Forest (drier types)	0.879 +/- 0.024	0.254 +/- 0.155
	Forest and secondary grassland	0.772 +/- 0.094	0.209 +/- 0.137
	Savannah	0.659 +/- 0.072	0.154 +/- 0.105
KI	East – North	0.897 +/- 0.041	0.209 +/- 0.138
	East – South	0.789 +/- 0.032	0.166 +/- 0.110
KII	East – North	0.495 +/- 0.151	0.256 +/- 0.165
	East – South	0.862 +/- 0.021	0.379 +/- 0.213
	Austral	0.720 +/- 0.069	0.108 +/- 0.080
	Cameroon	0.199 +/- 0.112	0.029 +/- 0.020

distribution from East to Central Africa via southern Africa. Finally, the clade *W* comprised 31 haplotypes and 139 individuals and was found only in West Africa (fig. 1). No haplotype was shared between West African populations and East-Central-Southern African populations. Both in MP and NJ analyses, *W* and *KI* were supported by high bootstrap values whereas *KII* was supported by lower bootstrap values. The phylogenetic relationships between these three major clades remain unresolved. The sister group status of clades *W* and *KI* was observed only in NJ analyses using the substitution models selected by hLRT and AIC criterion. In both NJ analyses, the bootstrap values remain low (55% and 56% respectively for hLRT and AIC).

Genetic structure of the *Busseola fusca* populations

Most of the molecular variation was accounted for by the differentiation between the three clades highlighted by phylogenetic analyses with Φ_{ST} = 0.868 (P < 10⁻⁵). At fine scale, the genetic structure was observed within local populations in each major clade. The different values were 0.226 (P < 10⁻⁵), 0.238 (P < 10⁻⁵), 0.344 (P < 10⁻⁵) respectively for *W*, *KI* and *KII* clades.

Diversity and demographic history of *Busseola fusca*

Haplotype and nucleotide diversity were calculated for different groups considered within each clade identified (tab. 1). The values of these indices vary greatly within each clade. For clade *W*, the forest region has the highest haplotype diversity. The East-North region is more diverse for clade *KI* than for other regions. Finally for *KII*, the East-South has the highest haplotype diversity. Both the variance (SSD)
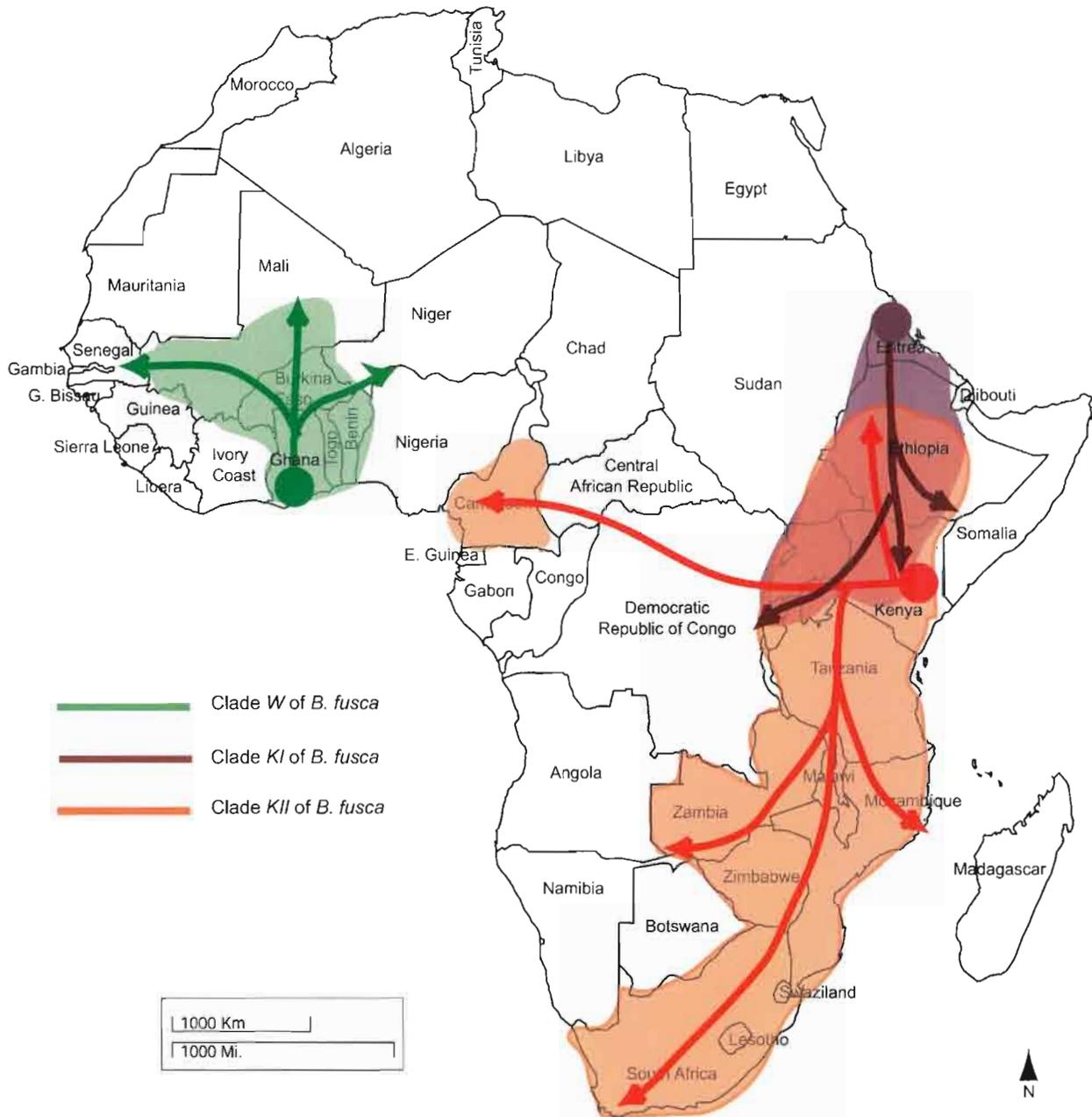
Figure 4
Current geographic distribution of *Busseola fusca* and the different putative centres of origin of its three main clades highlighted by phylogenetic analyses.

and raggedness index (r) tests suggested that the curves (figs 3a, 3b, 3c) do not significantly differ from the distribution under a model of population expansion ($P_{SSD} = 0.41$ and $P_r = 0.69$ for W; $P_{SSD} = 0.10$ and $P_r = 0.27$ for KI; $P_{SSD} = 0.87$ and $P_r = 0.94$ for KII). Similarly, the negative values obtained for Tajima's D index for each clade (-1.62105; -1.63863; -1.49025 for clades W, KI and KII, respectively) are all consistent with the hypothesis of population expansion since the origin of

the clades. The current geographic distribution of *B. fusca* with centre of origin of each clade is illustrated fig. 4.

Nested clade phylogeographic analysis

The NCPA network calculation identified the same three clades revealed by MP and NJ analyses. The networks of these three clades were represented by figs 5a, 5b, 5c. The West African clade contained the 31

Figure 5
Haplotype network of all haplotypes detected for *Busseola fusca*. Each haplotype is labelled by its number. Hypothetical haplotypes are designated by small circles (A: West Africa population; B: Kenya I population; C: Kenya II population).

haplotypes observed exclusively in this region and four hypothetical intermediate haplotypes, hierarchically grouped into 11 'one-step clades', four 'two-step clades', and two 'three-step clades'. The *KI* clade contained the 16 observed haplotypes and four hypothetical haplotypes: 8 'one-step clades' and 2 'two-step clades'. Finally, the *KII* clade, with 61 observed haplotypes and 10 hypothetical is organized into 20 'one-step' clades, 6 'two-step clades' and 2 'three-step clades'.

Nested contingency analysis on the haplotype network revealed significant geographic associations in the three major networks at all clade levels. These significant values were interpreted using Templeton's (2004) inference key (tab. 2). Most of the clades displaying geographic associations were interpreted by restricted gene flow with isolation by distance although some of them were interpreted as restricted gene flow or dispersal but with some long distance dispersal and one by contiguous range expansion.

Discussion

The goal of this study was to reconstruct the phylogeographic pattern of *Busseola fusca* across the whole geographic range of the species, to evaluate the current geographic distribution of each clade and to determine their centres of origin.

Genetic structure and phylogeographic pattern of *Busseola fusca*

All phylogenetic analyses confirm the separation of *B. fusca* into three major clades corresponding to three geographical units: one localized in West African region (*W*), one restricted to East Africa (*KI*) and one found from Central to East Africa via Southern Africa (*KII*). Partial geographic overlap was observed only between the clades *KI* and *KII*. The genetic distances between the clades suggest that the differentiation occurred during the Pleistocene (Sezonlin *et al.* 2006). Major climatic changes occurred in sub-Saharan Africa during the Pleistocene. The period of climatic instability started 3.3 to 2.45 Ma, oscillating between hot/humid and cooler, drier periods (Wagner 2002). A shift to arid, open conditions occurred in near 2.8 Ma, 1.7 Ma, and 1.0 Ma (de Menocal 1995). De Menocal (1995) concluded that this alternation of cold, dry periods and warmer, wetter periods led to oscillations in savannah biotope expansion. Pleistocene events also played an important role in differentiation of African vertebrates (Quérouil *et al.* 2003). The present study confirmed that *B. fusca* populations are differentiated in three clades that were isolated during Pleistocene in three different refuges (Sezonlin *et al.* 2006). This hypothesis was also supported by genetic structure analyses that

Table 2. Inference chain results of geographical distance analysis from Fig. 5a, 5b, 5c.

Clade	Chain of inference	Inference
1 – 1 (*W*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
1 – 2 (*W*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
1 – 4 (*W*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
1 – 6 (*W*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
1 – 10 (*W*)	1-2-11-17: NO	Inconclusive outcome
2 – 1 (*W*)	1-2-3-5-6-7: YES	Restricted gene flow / Dispersal but with some long distance dispersal
2 – 2 (*W*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
2 – 3 (*W*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
2 – 4 (*W*)	1-2-11-12: NO	Contiguous range expansion
3 – 2 (*W*)	1-2-3-5-6-7: YES	Restricted gene flow / Dispersal but with some long distance dispersal
1 – 2 (*KI*)	1-2-3-5-6-7: YES	Restricted gene flow / Dispersal but with some long distance dispersal
2 – 1 (*KI*)	1-2-3-5-6-7: YES	Restricted gene flow / Dispersal but with some long distance dispersal
1 – 3 (*KII*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
1 – 5 (*KII*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
1 – 18 (*KII*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
1 – 20 (*KII*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
2 – 2 (*KII*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
2 – 3 (*KII*)	1-2-11-17: NO	Inconclusive outcome
2 – 6 (*KII*)	1-2-3-5-6-7: YES	Restricted gene flow / Dispersal but with some long distance dispersal
3 – 1 (*KII*)	1-2-3-4: NO	Restricted gene flow with isolation by distance
3 – 2 (*KII*)	1-2-3-5-6-7: YES	Restricted gene flow / Dispersal but with some long distance dispersal

show high values of the fixation index between the three clades. The fragmentation hypothesis can be inferred between West African and other clades that do not overlap at all, but is questionable between the *KI* and *KII* clades that overlap in wide areas. According to Templeton *et al.* (1995), only mainly non-overlapping populations can clearly be inferred as a product of past fragmentation.

All the mitochondrial variation revealed significant and historical separation between clades *W*, *KI* and *KII*. The monophyly of the western populations allows the designation of at least one Evolutionary Significant Unit (ESU) (Alpers *et al.* 2004) within the *B. fusca* species. It is the same for *KI* and *KII* that can be considered as ESUs. If these clades are confirmed by nuclear polymorphism analyses (microsatellites

data), pheromones and behavioural ecology, therefore different biological strategies must be used to control *B. fusca* populations.

Origin and evolutionary history of each clade

In the present *B. fusca* study, the population of forest region has higher haplotype and nucleotide diversity for clade *W* than other phytogeographic regions of West Africa. It would likely be the centre of origin for this clade. Alpers *et al.* (2004) found also a center of origin in Ghana for the West African populations of the roan antelope (*Hippotragus equinus*) (Desmarest 1804), a herbivorous species that has a sub-Saharan distribution similar to that of *B. fusca*. Concerning the *KI* clade, the eritrean region, where only *KI* individuals were found was more diverse than other regions. Accordingly, we will consider it as the possible/putative centre of origin for clade *KI*. Finally for clade *KII*, the highest haplotype and nucleotide diversity was found among population of South-East region. The centre of origin of this clade is likely in this geographic area. Consequently, we can suggest that the centres of origin were likely localized in forest, East-North and East-South regions respectively for clades *W*, *KI* and *KII*. We observed that the strongest haplotype diversity was associated with the lowest levels of nucleotide diversity. This accumulation of haplotypes suggests that the clades experienced bottlenecks at their origins, followed by major population demographic expansion (Grant & Bowen 1998; Avise 2000). However, Petit *et al.* (2003) have shown that for some European tree species the highest diversity is not observed in the centre of origin but rather in secondary contact zones. Therefore in *B. fusca* case, further studies will be necessary to confirm the reality of these possible centres of origin. The fact that clades *KI* and *KII* originate from East Africa gives support to some previous studies (Livingstone 1982, Arctander *et al.* 1999). Indeed, these authors have described the East African region as a mosaic of secondary refuge zones for herbivorous mammals, with periodic exchanges between refuge zones through temporary contact bridges in the East African Rift Valley. Although the East African populations of *B. fusca* are now overlapping, the Rift Valley was pointed out as one of the main factors that explain most of the molecular variation in East Africa (Sezonlin *et al.* 2006). The Rift Valley seems to act as an important natural barrier to maintain population structure for other African species in East Africa (Arctander *et al.* 1999, Pitra *et al.* 2002).

The current distribution of *B. fusca* populations can be explained by contiguous range expansion or by dispersal with some long distance dispersal as it is highlighted by NCPA inferences. This pattern is probably linked to the expansion of its wild host plant (*Sorghum arundinaceum* (Desv. Stapf) (Poaceae) (Haile & Hofsvang 2002) during the Pleistocene. The presence of individuals of the large population unit, clade *KII* in Central Africa (Cameroon) is consistent with the hypothesis of a faunistic link between these two regions (Bruhl 1997), which are separated by a distance of 3000 km. An eastern origin of central populations is a possibility as it was suggested for a butterfly species in Cameroon (De Jong & Congdon 1993). De Jong & Congdon (1993) argued that the low animal species diversity in highland forests of Cameroon suggests that these species originated from long distance migration from East Africa. The faunistic link between Eastern and Central Africa also exists for some vertebrate species (Pitra *et al.* 2002). However, the nature of this faunistic link was not elucidated by our study and remains unknown for *B. fusca*. The present study shows that the clade *KII* is also present in Southern Africa. This geographic expansion toward Southern Africa is consistent with the patterns highlighted by the study of some African vertebrates (Faulkes *et al.* 2004). Climatic and topographic differences between major biogeographic African regions (de Menocal 1995) might explain the different processes that govern the current geographic distribution of each *B. fusca* clade.

Evolution of host plant specialization in *Busseola fusca*

Many ecological studies have shown that the introduction of exotic plants can lead to host plant shifts in oligo- or monophagous insect species. This is the case for *Rhagoletis pomonella* (Walsh 1867) (Diptera: Tephritidae) which in North America switched from hawthorn to introduced apple tree (Bush 1994); the bug *Jadera haematoloma* (Herrich-Schaeffer 1847) (Hemiptera: Rhopalidae), which added to its host plant spectrum an introduced ornamental plant of the family Sapindaceae (Carroll & Boyd 1992). Other examples of such host switches are the nymphalid *Euphydrias editha* (Boisduval 1852) (Lepidoptera: Nymphalidae) which switched to *Plantago lanceolata* L. (Plantaginaceae), introduced by North American breeders (Singer *et al.* 1993), the groundnut beetle *Caryedon serratus* (Olivier 1790) (Coleoptera: Bruchidae) which added groundnut, *Arachis hypogea* L. (Fabaceae), an introduced Papilionoideae to its native host plant range (Delobel 1995) and finally African cereal stem borers such as *B. fusca* and *Sesamia calamistis* Hampson 1910 (Lepidoptera, Noctuidae) which shifted to maize after its introduction into Africa.

Host plant shifts appear as a major factor promoting ecological specialization in phytophagous insects.

Within an insect population on a given host plant, some genotypes procuring better fitness to their bearers can be selected. This phenomenon may occur within the various populations of an insect species associated with different host plants and leads to genetic differentiation between these populations. A study by Via *et al.* (2000) on the aphid *Acyrthosiphon pisum* (Harris 1776) (Hemiptera: Aphididae) that feed on two different legume species shows that direct selection against migrants and hybrids in each parental environment could favour the evolution of more precise or efficient habitat choices. Moreover, these authors argued that the differential phenology of host plants strengthens ecological specialization by increasing the reproductive isolation between populations. This phenomenon promotes mating within the same habitat. As is well established for some phytophagous insects, such host plant shifts can lead to ecological segregation of populations with appearance of host races and genetic differentiation. This is well known with *R. pomonella* (Feder *et al.* 1988; McPheron *et al.* 1988), *R. cerasi* (L. 1758) (Diptera: Tephritidae) (Schwartz *et al.* 2003), the European corn borer *Ostrinia nubilalis* (Hübner 1796) (Lepidoptera: Pyralidae) (Thomas *et al.* 2003), *Spodoptera frugiperda* (Smith & Abbott 1797) (Lepidoptera: Noctuidae) (Pashley Prowell *et al.* 2004), *C. serratus* (Sembène 2000) and the pea aphid *A. pisum* (Via *et al.* 2000; Caillaud & Via 2000). Genetic differentiation of such host races can occur within a limited period of time. For example, it has taken a few centuries for *R. pomonella* and *C. serratus* populations to differentiate, several tens of years for *J. haematoloma* and less than 20 years for *E. editha*. Considering the age of sorghum domestication and of maize introduction in Africa, it is thus likely that these major agricultural events could have been at the origin of such a phenomenon of genetic divergence among *B. fusca* populations and possibly at the origin of host race appearance in this species. The fact that *B. fusca*, considered as oligophagous (Le Rü *et al.* 2006) now preferentially uses maize and cultivated sorghum in most part of its distribution areas suggests that these host plants offer this species very suitable resources. The preference for these cultivated plants (Kfir *et al.* 2002, Le Rü *et al.* 2006) would represent one of the factors that might have led *B. fusca* to start ecological specialization between populations. This ecological segregation between *B. fusca* populations exploiting wild and cultivated host plants or cultivated sorghum and maize could be highlighted by further molecular studies. However, a preliminary population genetic study using mitochondrial marker (Sezonlin *et al.* 2006) has not demonstrated any genetic structure between cultivated sorghum and maize *B. fusca* populations. An extensive survey carried out in East and West Africa indicates that *B. fusca* is now mostly associated with maize and sorghum crops and is generally uncommon in the wild habitat (Le Rü *et al.* 2006; Sezonlin *et al.* unpublished) Therefore, we have not been able until now to test ecological segregation between wild host plants and cereal crops, but the recent discovery of *B. fusca* populations associated to *Phragmites mauritianus* Kunth. (Poacaea) in Ethiopia and Eritrea and to *Setaria megaphylla* (Steud.) (Poaceae) T. Duran & Schinz in Kenya (Le Rü *et al.* 2006) will soon help us to test this hypothesis.

References

Ajayi O. **1998.** Sorghum: West Africa, p. 39-45 *in*: **Polaszek A. (ed.)**, *African cereal stem borers: economic importance, taxonomy, natural enemies and control.* CABI, Wallingford, UK.

Alpers D.L., Van Vuuren B.J., Arctander P., Robinson T.J. **2004.** Population genetics of the roan antelope (*Hippotragus equinus*) with suggestions for conservation. *Molecular Ecology* **13**: 1771-1784.

Althoff M.D., Pellmyr O. **2002.** Examining genetic structure in bogus yucca moth: A sequential approach to phylogeography. *Evolution* **56**: 1632-1643.

Arctander P., Johansen C., Coutellec-Vreto M. **1999.** Phylogeography of three closely related African bovids (Tribe *Alcelaphini*). *Molecular Biology and Evolution* **16**: 1724-1739.

Avise J.C. **2000.** *Phylogeography. The History and Formation of Species.* Harvard University Press, Cambridge, Massachusetts, 447 p.

Bernatchez L. **2001.** The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution* **55**: 351-379.

Bruhl C.A. **1997.** Flightless insects: a test case for historical relationships of African mountains. *Journal of Biogeography* **24**: 233-250.

Bush G.L. **1994.** Sympatric speciation in animals: new wine in old bottles. *Trends in Ecology and Evolution* **9**: 285-288.

Caillaud C.M., Via S. **2000.** Specialized feeding behavior influences both ecological specialization and assortative mating in sympatric host races of pea aphids. *The American Naturalist* **156**: 607-621.

Carroll S.P., Boyd C. **1992.** Host race radiation in the soapberry bug: Natural history with the history. *Evolution* **46**: 1052-1069.

Chastanet M. **1998.** Introduction et place du maïs au Sahel Occidental (Sénégal-Mauritanie), p. 251-275 *in*: **Chastanet M. (ed.)**, *Plantes et paysages d'Afrique. Une histoire à explorer.* Karthala-CRA, Paris.

Clement M., Posada D., Crandall K.A. **2000.** TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657-1660.

De Jong R., Congdon T.C.E. 1993. The montane butterflies of the eastern Afrotropics, p. 133-164 in: Lovett J. C., Wasser S. K. (eds.), *Biogeography and ecology of the rain forests of eastern Africa*. Cambridge University Press, Cambridge.

Delobel A. 1995. The shift of *Caryedon serratus* (Ol.) from wild Caesalpiniaceae to groundnuts took place in West Africa (Coleoptera: Bruchidae). *Journal of Stored Products Research* 31: 101-102.

Doggett H. 1988. *Sorghum*. 2nd ed, Longman Scientific and Technical Publishers, London, 512 p.

Excoffier L., Smouse P.E., Quattro J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Faulkes C.G., Verheyen E., Verheyen W., Jarvis J.U.M., Bennett N.C. 2004. Phylogeographical patterns of genetic divergence and speciation in African mole-rats (Family: Bathyergidae). *Molecular Ecology* 13: 613-629.

Feder J.L., Chilcote C.A., Bush G.L. 1988. Genetic differentiation between sympatric host races of the apple maggot fly, *Rhagoletis pomonella*. *Nature* 336: 61-64.

Grant S.W., Bowen B.W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* 89: 415-426.

Gu X., Fu Y.X., Li W.H. 1995. Maximum likelihood estimation of heterogeneity of substitution rate among nucleotide sites. *Molecular Biology and Evolution* 12: 546-557.

Haile A., Hofsvang T. 2001. Survey of lepidopterous stem borers of sorghum, maize and pearl millet in Eritrea. *Crop Protection* 20: 151-157.

Haile A., Hofsvang T. 2002. Host plant preference of the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Crop Protection* 21: 227-233.

Hasegawa M., Kishino H., Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 21: 160-174.

Holloway J. D. 1998. Noctuidae, p. 79-86 in: Polaszek A. (ed), *African cereal stem borers: economic importance, taxonomy, natural enemies and control*. CABI, Wallingford, UK.

Kfir R., Overholt A.W., Khan R.Z., Polaszek A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology* 47: 701-731.

Kimura M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences of the USA* 78: 454-458.

Le Rü B.P., Ong'amo G.O., Moyal P., Muchugu E., Ngala L., Musyoka B., Abdullah Z., Kauma-Matama T., Lada V.Y., Pallangyo B., Omwega C.O., Schulthess F., Calatayud P.-A., Silvain J.-F. 2006. Geographic distribution and host plant ranges of East African noctuid stem borers. *Annales de la Société Entomologique de France* 42(3-4): 353-361.

Livingstone D.A. 1982. Quaternary geography of Africa and refuge theory, p. 523-536 in: Prance G. T. (ed.), *Biological diversification in the tropics: Proceedings of the 5th international symposium of the association for tropical biology*. Columbia University Press, New York.

Maddison D.R., Maddison W.P. 2002. *MacClade: Analysis of phylogeny and character evolution*. Version 4.02. Sinauer Associates, Sunderland, Massachusetts.

Madeiras Santos M.E., Ferraz Torrao M.M. 1998. Entre l'Amérique et l'Afrique, les îles du Cap-vert et Sao Tomé: les cheminements des milhos (mil, sorgho et maïs), p. 69-83 in: Chastanet M. (ed.), *Plantes et paysages d'Afrique. Une histoire à explorer*. Karthala-CRA. Paris.

McPheron B.A., Smith C.D., Berlocher S.H. 1988. Genetic differences between host races of *Rhagoletis pomonella*. *Nature* 336: 64-66.

Menocal P.B. de 1995. Plio-Pleistocene African climate. *Science* 270: 53-59.

Moyal P. 1998. Infestation patterns and parasitism of the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in the Ivory Coast. *African Entomology* 6: 289-296.

Murty D.S., Renard C. 2001. Sorgho (*Sorghum bicolor* (L.) Moench), p. 105-125 in: Raemaekers R. H. (ed.), *Agriculture en Afrique Tropicale*. Direction Générale de la Coopération Internationale, Bruxelles, Belgique.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Cardwell K.F. 2001. Distribution, relative importance and effect of lepidopterous borers on maize yields in forest zone and mid-altitude of Cameroon. *Journal of Economic Entomology* 94: 1434-1444.

Nei M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York, 512 p.

Nei M., Li W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA* 76: 5269-5273.

Pashley Prowell D., McMichael M., Silvain J.-F. 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains fall armyworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* 97: 1034-1044.

Petit R.J, Aguinagalde I., de Beaulieu J-L., Bittkau C., Brewer S., Cheddadi R., Ennos R., Fineschi S., Grivet D., Lascoux M., Mohanty A., Müller-Starck G., Demesure-Musch B., Palmé A., Martín J.P., Rendell S., Vendramin G.G. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* 300: 1563-1565.

Pitra C., Hansen J.A., Lieckfeldt D., Arctander P. 2002. An exceptional case of historical outbreeding in African sable antelope populations. *Molecular Ecology* 11: 1197-1208.

Polaszek A., Khan Z.R. 1998. Host plants, p. 3-10 in: Polaszek A. (ed.), *African cereal stem borers: economic importance, taxonomy, natural enemies and control*. CABI, Wallingford, UK.

Posada D., Crandall K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Posada D., Crandall K.A., Templeton A.R. 2000. GeoDis: A program for the cladistic nested analysis of geographical distribution of genetic haplotypes. *Molecular Ecology* 9: 487-488.

Quérouil S., Verheyen E., Dillen M., Colyn M. 2003. Patterns of diversification in two African forest shrews: *Sylvisorex johnstoni* and *Sylvisorex ollula* (Soricidae, Insectivora) in relation to paleo-environmental changes. *Molecular Phylogenetics and Evolution* 28: 24-37.

Schneider S.D., Excoffier L. 1999. Estimation of past demographic parameters from the distribution of pairwise distances when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152: 1079-1089.

Schneider S.D., Roessli D., Excoffier L. 2000. *Arlequin V. 2.000: a software for genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Schwartz D., McPheron A.B., Hartl B.G., Foller F.E., Hoffmeister S.T. 2003. A second case of genetic host races in *Rhagoletis*? A population genetic comparison of sympatric host populations in the European cherry fruit fly, *Rhagoletis cerasi*. *Entomologia Experimentalis et Applicata* 108: 11-17.

Sembène M. 2000. *Variabilité de l'Espaceur Interne transcrit (ITS1) de l'ADN ribosomique et polymorphisme des locus microsatellites chez la bruche d'arachide Caryedon serratus (Olivier) : différenciation en races d'hôtes et infestation de l'arachide au Sénégal*. Thèse de doctorat d'Etat ès sciences, Université Cheik Anta Diop de Dakar, Dakar, 214p.

Sezonlin M., Dupas S., Le Rü B., Le Gall P., Moyal P., Calatayud P.-A., Giffard I., Faure N., Silvain J.-F. 2006. Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa. *Molecular Ecology* 15: 407-420.

Simmons R.B., Weller S.J. 2001. Utility and evolution of cytochrome *b* in insects. *Molecular Phylogenetics and Evolution* 20: 196-210.

Singer M.C., Thomas C.D., Parmesan C. 1993. Rapid human-induced evolution of insect-host associations. *Nature* **366**: 681-683.

Slatkin M., Hudson R.R. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* **129**: 555-562.

Swofford D.L. 2002. PAUP*. *Phylogenetic Analysis Using Parsimony (*and other methods)*. Sinauer Associates, Sunderland, Massachusetts.

Tajima F. 1989a. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585-595.

Tajima F. 1989b. The effect of change in population size on DNA polymorphism. *Genetics* **123**: 597-601.

Templeton A.R., Routman E., Phillips C.A. 1995. Separating population structure from population history: a cladistic analysis of the geographical dispersion of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum. Genetics* **140**: 767-782.

Templeton A.R. 1998. Nested clade analysis of phylogeographic data: testing hypothesis about gene flow and population history. *Molecular Ecology* 7: 381-397.

Templeton A.R. 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. *Molecular Ecology* **13**: 789-809.

Thomas Y., Bethenod M.T., Pelozuelo L., Frérot B., Bourguet D. 2003. Genetic isolation between two sympatric host-plant races of the European corn borer, *Ostrinia nubilalis* Hübner. I. Sex pheromone, moth emergence timing, and parasitism. *Evolution* **57**: 261-273.

Via S., Bouk C.A., Skillman S. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution* **54**: 1626-1637.

Wagner T. 2002. Late Cretaceous to early Quaternary organic sedimentation in the eastern Equatorial Atlantic. *Palaeogeography, Palaeoclimatology, Palaeoecology* **179**: 113-147.

White F. 1983. The vegetation of Africa, a descriptive memoir to accompany the UNESCO / AETFAT / UNSO vegetation map of Africa. *UNESCO, Natural Resources Research* **20**: 1-356.

Yang Z. 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* **10**: 1396-1402.

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Geographic distribution and host plant ranges of East African noctuid stem borers

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* DGIS funded Stem borer Biological Control NETwork for ESA (SBCNET)

Abstract. Surveys were carried out in Kenya, Tanzania, Uganda and Zanzibar to establish geographic distribution in the main vegetation mosaics and ecological (host plant range, feeding behaviour) characteristics of the East African noctuid stem borers. 49 wild plant species belonging to Poaceae, Cyperaceae and Typhaceae were found to harbour stem borers in the six vegetation mosaics surveyed. A total of 36 noctuid species belonging to nine genera were identified from 14,318 larvae collected, out of which 17 were new to science. The species diversity varied among vegetation mosaics and host plants. Most borer species appeared to be specialised feeders with 24 species being monophagous. Species belonging to the same types (named as the *Busseola* Thurau 1904 and the *Sesamia* Guenée 1852 types) or genus harboured common ecological characteristics such as pigmentation and feeding site. The *Sciomesa* Tams and Bowden 1953 genus was an exception as it had a mixture of these characters.

Résumé. Distribution géographique et spectre d'hôtes des foreurs de graminées d'Afrique de l'Est. Des enquêtes ont été conduites au Kenya, en Tanzanie, en Ouganda et à Zanzibar afin de connaître les caractéristiques écologiques des noctuelles foreuses africaines tels que le spectre d'hôtes, le mode de nutrition et les principales caractéristiques de développement. 49 espèces de plantes appartenant aux familles des Poaceae, Cyperaceae et Typhaceae ont été trouvées infestées par des foreurs dans les six phytochories rencontrées. Sur un total de 14318 chenilles récoltées, nous avons identifié 36 espèces de noctuelles appartenant à neuf genres. 17 espèces nouvelles ont été trouvées. La diversité spécifique varie d'une phytochorie à l'autre et d'une plante hôte à l'autre. La plupart des espèces de foreurs ont été trouvées dans les zones humides des phytochories et présentent un comportement alimentaire spécialisé : 24 espèces sont monophages, et la plupart des huit espèces oligophages montrent une préférence marquée pour une ou deux plantes.

Keywords: Stem borer, Poaceae, feeding behaviour, vegetation mosaic, Africa.

During the last 50 years, several surveys have been carried out in Kenya, Uganda and Tanzania to identify wild host plants of noctuid stem borers (Noctuidae) (Ingram 1958; Nye 1960; Seshu Reddy 1989; Randriamananoro 1996; Polaszek & Khan 1998). Ten noctuid species (two *Busseola* Thurau 1904,

seven *Sesamia* Guenée1852, one *Poeonoma* Tams and Bowden 1953) were recovered from 34 wild host plants. It is expected that the list of host plants of these 10 species is by far not exhaustive because the surveys were short, carried out in a limited area and mostly on crops (Ingram 1958; Nye 1960; Seshu Reddy 1989; Polaszek & Khan 1998). The most damaging species in eastern Africa, *Busseola fusca* (Fuller 1901) and the less important species *Sesamia calamistis* Hampson 1910 were recovered from 25 and 28 host plants, respectively, and it was suggested that these species were polyphagous (Polaszek & Khan 1998).

B. P. Le Rü, G. O. Ong'amo, P. Moyal, E. Muchugu, L. Ngala, B. Musyoka, Z. Abdullah, T. Matama-Kauma,
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Noctuid stem borers characteristically feed inside the stems of grasses (Poaceae), sedges (Cyperaceae) and bulrush (Typhaceae). They are a very diverse group and over half of the 190 known tropical species are from Africa (Holloway 1998). 32 were reported from East Africa, among them eight *Acrapex* Hampson 1894, two *Busseola*, two *Manga* Bowden 1956 , one *Poecopa* Bowden 1956, one *Poeonoma*, five *Sciomesa* Tams and Bowden 1953, and 13 *Sesamia* spp. However, only hosts of the 10 species mentioned in the previous paragraph are known. The remaining 22 species were obtained as adults from light traps. Their description was based on scanty materials (one to three specimens) and frequently only on one sex. (Fletcher 1961; Rougeot 1984). As a result, the boring behaviour could not be verified for some genera such as *Acrapex, Sciomesa* and *Speia* Tams and Bowden, 1953.

To understand how this highly specialised phytophagous moth group has evolved is not an academic luxury for evolutionists and entomologists. It might be predictive as groups of taxa descending from a common ancestor are more likely to share common biological features (i.e. rapid population increase, short generation time), host plants, behavioural characteristics and common natural enemies (Holloway 1998). Current studies on how phytophagous insect-plant associations evolved combine studies of systematics with ecological and biogeographical data (Johnson 1990; Wahlberg 2001; Kergoat *et al.* 2004). Therefore, detailed knowledge on geographic distribution in the main vegetation mosaics, ecology (host plant ranges, feeding site) and interactions with other stem borers (i.e. guild diversity of the exploited host plants)), should contribute to future investigations on noctuid stem borer-plant evolutionary history.

The present work is an attempt to address the above principles in the study through extensive surveys in wild habitats surrounding cereal fields in Uganda, Kenya, Tanzania and Zanzibar, and to appraise the natural distribution of noctuid stem borers and associated host plant species in East Africa. It also provides some basic information necessary for future studies on the evolution of this moth group.

Materials and Methods

Selection of localities and description of the vegetation mosaics

Surveys were conducted in the major cereal growing areas in Kenya between January 2003 and April 2005, Uganda in April 2004 and March 2005, Tanzania in June 2004 and February 2005 and in Zanzibar in May 2004. A total of 158 localities distributed in the six main vegetation mosaics (described and numbered by White 1983) encountered in East Africa were visited. The vegetation mosaics included the Guineo-Congolian mosaic [mosaic of lowland rain forest and secondary grassland (Mosaic no. 11)], the Zanzibar-Inhambane mosaic [East African coastal mosaic (No. 16)], the Afromontane mosaic [undifferentiated montane vegetation (No. 19)], the Zambezian woodland mosaic [drier Zambezian miombo woodland dominated by *Brachystegia* and *Julbernardia* (No. 26)], the Somalia-Masai mosaic [Somalia-Masaï *Acacia-Commiphora* deciduous bushland and thicket (No. 42)] and the East African mosaic [mosaic of East African evergreen bushland and secondary *Acacia* wooded grassland (No. 45)] (tab. 1, fig. 1). Localities within the mosaics were selected on the basis of accessibility and presence of potential host plants. Each locality was referenced with its geographic coordinates (latitude, longitude and altitude) using a GARMIN 12X portable Geographic Positioning System (GPS). The localities ranged between sea level to 2396 m above sea level (m.a.s.l.), 1°15' North and 08°32' South and, 29°43' and 39°32' East. The climatic conditions found in the mosaics were sourced from Africa AWhere-ACT Database (2002) and summarized in Table 1.

Collection and rearing of stem borer materials

In each locality, the plant habitats (i) in and around crops, (ii) in open patches along forest roads, (iii) on banks of streams or rivers and (iv) in swamps were checked for stem borer infestation. Because noctuid borer densities on wild hosts are exceedingly low (Nye 1960; Gounou & Schulthess 2004), a biased rather than a random sampling procedure was use to increase the chances of finding borers. In all habitats, plant species belonging to Poaceae, Cyperaceae and Typhaceae families were carefully inspected for stem borer infestation symptoms or damage [scarified leaves, dry leaves and shoots (dead hearts), frass, holes bored]. Number of visits per locality varied between one (some localities from Uganda, Tanzania and Zanzibar) and nine (some Kenyan localities), and time spent examining host plants in a locality varied from one to four hours with two or three people depending of the infestation levels. Infested plants were cut and dissected in the field. The kind and location of damage were recorded for all plants found infested. The species collected were then grouped into the *S. calamistis* type and the *B. fusca* type. In the *S. calamistis* type the neonates fed on the basal leaf sheaths for 2-3 days, after which they rapidly penetrated into the stem. In the *B. fusca* type, the first and second instar larvae fed on young leaves (whorl) of the main stem producing the typical "window" damage, or on the flower spikes (Ingram 1958, Nye 1960). The number of young larvae (1st, 2nd, 3rd instars), old larvae (4th, 5th and 6th instars), pupae and, the colour and adornment of the larvae were also recorded (WDS: white with dark thin stripes, B: buff, BLPB: buff with longitudinal wide pale bands, BP: pink, DG: dark grey, OPR: olive with purple rings). Recovered pupae were kept in plastic vials (15 x 7 cm) closed with perforated plastic lids until emergence. Recovered larvae were all reared until pupation on artificial diet (Onyango & Ochieng-Odero 1994) kept in glass vials (7.5 x 2.5 cm) closed with cotton wool or fresh maize stems (10 cm long) kept in plastic vials (16 x 10 cm) with perforated plastic lids. Pupae taken out of the medium/ maize stems were kept separately in plastic vials (16 x 10 cm) closed with perforated plastic lids until adult emergence.
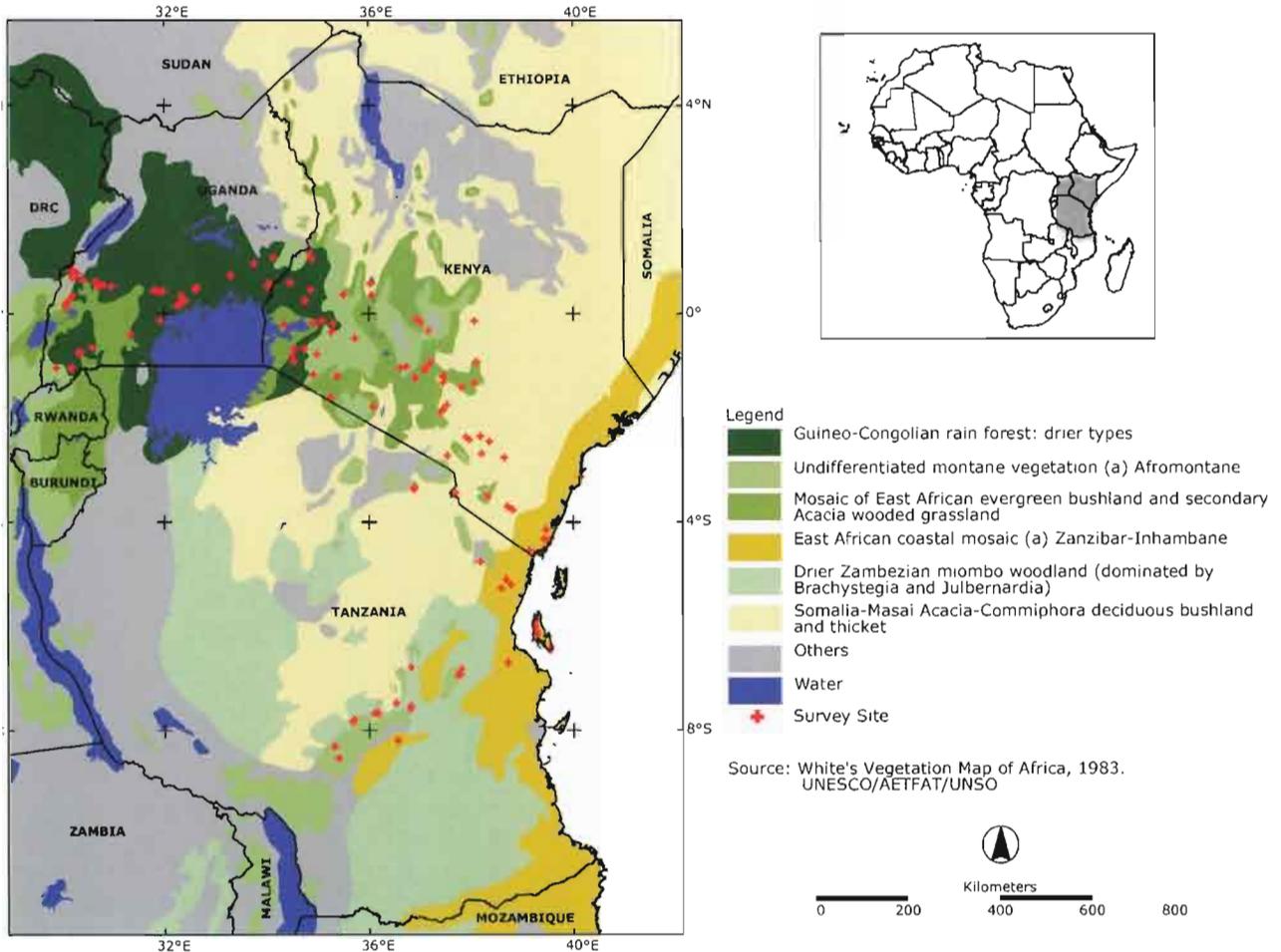
Figure 1
White's vegetation mosaic map of East Africa, 1983, Unesco/AETFAT/UNSO.

Table 1. Average climatic conditions of the different vegetation mosaics found in East Africa (from Africa AWhere-ACT database 2002).

Mosaic No	Rainfall (mm)	Moisture index	Min temp (°C)	Max temp (°C)	Altitude (m.a.s.l.)
11	961–1511 (1237)	0.64–1.05 (0.84)	12.05–17.04 (15.24)	26.10–29.40 (27.52)	699–2160
16	900–1330 (1102)	0.60–0.90 (0.77)	20.48–22.02 (21.22)	29.00–31.15 (30.31)	20–2130
19	669–1621 (1162)	0.46–1.26 (0.88)	06.46–18.42 (12.38)	18.89–29.28 (24.61)	851–2396
26	501–1337 (746)	0.33–1.00 (0.49)	11.90–20.19 (16.72)	22.46–32.95 (28.93)	479–1460
42	886–1304 (1081)	0.63–0.87 (0.74)	13.81–19.70 (17.32)	24.31–30.26 (27.50)	484–1754
45	597–1242 (940)	0.34–1.00 (0.63)	10.09–18.96 (14.44)	23.11–31.83 (27.23)	781–1956

Identification of moths and analysis of borer species diversity

Adult moths were identified to species level (Moyal, P.) and voucher specimens deposited in Museum National d'Histoire Naturelle (MNHN, Paris, France) and in ICIPE Museum (Nairobi, Kenya).

The identified borers were then grouped in their respective host plant and vegetation mosaic for analysis of species diversity. The data from each of the vegetation mosaics and from each host plants were then used to compute diversity indices described by Samways (1984) (tab. 2).

Species richness (S). This is based on presence, rather than relative abundance. This conceptually easy index is widely used both in ecology and biogeography.

Berger-Parker dominance index (d). This index uses the abundance of the dominant species relative to the abundance of all species together.

Fisher-Williams diversity index (α). This useful statistic is based on the log-series. It is particularly valuable if N>1000 because of its lack of sensitivity to the greatly fluctuating common species. It is a parametric index that describes the relationship between the number of species and the number of individuals in those species.

Rare species are defined as those recovered from only one locality.

Results

Vegetation mosaic

Results on noctuid stem borer species found in the 6 vegetation mosaics surveyed are summarized in Table 1. The total number of noctuid larvae collected ranged between 683 and 4094 in the dry and hot Zambezian woodland and cool afromontane mosaics, respectively. The highest species diversity (S = 22; α = 3.22) was recorded in the wet and hot Guineo-Congolian mosaic. Proportions of the dominant species varied between 0.26 in the Somalia-Masai mosaic and 0.81 [(Berger-Parker indices (*d*)] in the Zambezian mosaic. *Manga nubifera* Hampson 1910 was dominant in both Somalia-Masaï (*d* = 0.26) and East African bushland (*d* = 0.27) mosaics, while *Manga*

melanodonta Hampson 1910 and *Busseola s.l. n. sp. 1* respectively dominated Guineo-Congolian (*d* = 0.33) and Afromontane (*d* = 0.30) mosaics. *Sesamia* n. sp. 5 dominated in Zanzibar-Inhambane (*d* = 0.51) and Zambezian miombo (*d* = 0.81) (tab. 1). Rare species were found in five vegetation mosaics, contributing 12 (33%) out of the total of 36 species collected (tab. 3). No rare species were found in the dry Zambezian woodland mosaic. The Afromontane mosaic alone harboured 42% of the rare species accounting for 28% of the total species recovered in this vegetation mosaic. In the other vegetation mosaics the proportion of rare species ranged between 6% (East African mosaic) and 37% (Zanzibar-Inhambane).

Host-plants

Noctuid stem borers were recovered from 49 plant species belonging to three families: Poaceae (35), Cyperaceae (13) and Typhaceae (1) (tab. 4). Out of the 14,318 larvae collected, 92.2% were from Poaceae, 3.4% from Cyperaceae and 4.4% from Typhaceae. 63.5% of the larvae belonging to 22 noctuid species of seven genera were collected from three species of Poaceae, namely *Pennisetum purpureum* Schumacher, *Panicum maximum* Jacquin and *Setaria megaphylla* (Steudel) Th. Durand & Schinz. Borer species richness within the host-plants varied between one (22 host plants) and 11 (*P. purpureum*) with α value varying between 0.14 (*Cynodon aethiopicus* Clayton & Harlan) and 1.59 (*Cyperus latifolius* Poiret).

Noctuid species

The 14,318 noctuid larvae/pupae collected belonged to 36 species, out of which one belonged to the genus *Acrapex*, three *Busseola*, three *Carelis* Bowden 1956, two *Manga*, one *Poeonoma*, nine *Sciomesa*, twelve *Sesamia*, one *Speia* and four species related to *Busseola*, temporarily named as *Busseola s.l.* (tab. 3). Seventeen out of the 36 species collected are new to

Table 2. Stem borer species diversity in respective vegetation mosaics.

Veg. Mosaic associations (N localities)	Descriptive values			Statistics		
	Individual abundance	Species richness (S) (Endemic species: N/%)	α	Berger-Parker (*d*)	Dominant species
11 (43)	3046	22 (5/23)	3.22	0.33	*Manga melanodonta*
16 (16)	1595	8 (3/37)	1.10	0.51	*Sesamia* n. sp. 5
19 (20)	4094	18 (6/33)	2.45	0.30	*Busseola s.l.* n. sp.1
26 (9)	683	6 (0/0)	1.09	0.81	*Sesamia* n. sp. 5
42 (20)	3003	14 (1/7)	1.75	0.26	*Manga nubifera*
45 (20)	1897	16 (1/6)	2.58	0.27	*Manga nubifera*

Table 3. Noctuid stem borer species recorded from East Africa.
* : new borer species, Ns: Borer species number, IA: Individual Abundance (Y : Young larvae, O : Old larvae, P : Pupae) , NL: Number of Localities, WDS: white with dark thin stripes, B: buff , BLPB: buff with longitudinal wide pale bands, BP: pink, DG: dark grey, OPR: olive with purple rings, FL: young instars feed on leaves, BS: young instars bore straight into the stems.

Ns	Noctuid stem borer species	IA (Y/O/P)	NL	Host plants (see tab. 4)	Vegetation mosaics	Mature larvae colour	Feeding behaviour
	Acrapex						
1	A. syscia Fletcher	90 (8/82/0)	1	34	45	P	BS
	Busseola						
2	B. fusca Fuller	294 (104/185/5)	6	2, 26, 31, 33	11, 45	B	FL
3	B. phaia group	1241 (689/537/15)	31	4, 7, 10, 15, 17, 18, 19, 25, 26, 29, 32, 33	11, 19, 42,45	BLPB	FL
4	Busseola n. sp. 1*	185 (45/137/3)	6	19, 27	11, 19, 26	BLPB	FL
	Busseola s.l.						
5	Busseola s.l. n. sp. 1*	1197 (674/498/25)	3	31	11, 19	WDS	FL
6	Busseola s.l. n. sp. 2*	91 (48/43/0)	1	31	19	OPR	FL
7	Busseola s.l. n. sp. 3*	447 (69/335/33)	7	4, 10, 18, 23, 27, 28	11, 19, 45	WDS	FL
8	Busseola s.l. n. sp. 4*	40 (5/34/1)	1	31	19	?	?
	Carelis						
9	Carelis n. sp. 1*	39 (0/39/0)	1	39	19	P	BS
10	Carelis n. sp. 2*	164 (15/149/0)	1	31	19	P	BS
11	Carelis n. sp. 3*	17 (0/15/2)	1	48	11	P	BS
	Manga						
12	M. melanodonta Hampson	1440 (949/491/0)	8	17, 18, 19, 24, 31, 32, 33	11, 42, 45	DG	BS
13	M. nubifera Hampson	2043 (1463/580/0)	12	18, 19	11, 16, 26, 42, 45	DG	BS
	Poeonoma						
14	P. serrata Hampson	995 (928/64/3)	11	25, 26	11, 19	DG	FL
	Sciomesa						
15	Sc. argocyma Fletcher	11 (0/9/2)	1	38	19	P	BS
16	Sc. mesophaea Aurivillius	335 (24/302/9)	9	36, 37, 39, 41, 44, 49	11, 19, 42, 45	P	FL
17	Sc. nyei Fletcher	658 (538/119/1)	4	27	11, 19	WDS	BS
18	Sc. piscator Fletcher	1179 (184/995/0)	27	3, 7, 9, 10, 12, 13, 23, 25, 26, 27, 29, 30, 36, 41, 43, 46, 47, 48	11, 19, 26, 42, 45	P	BS
19	Sc. venata Fletcher	34 (2/32/0)	1	36	11	P	BS
20	Sciomesa n. sp. 1*	58 (16/42/0)	2	31	19	WDS	BS
21	Sciomesa n. sp. 2*	78 (47/31/0)	2	17	42, 45	P	FL
22	Sciomesa n. sp. 3*	2 (0/2/0)	1	17	42	P	?
23	Sciomesa n. sp. 4*	183 (128:55/0)	6	5	42, 45	WDS	BS
	Sesamia						
24	S. calamistis Hampson	354 (197/145/12)	24	6, 12, 18, 21, 22, 26, 29, 30, 33, 35, 36, 37, 40, 41, 42, 43, 44, 45, 46	11, 16, 19, 42, 45	P	BS
25	S. jansei Tams & Bowden	27 (0/25/2)	1	36	16	P	BS
26	S. nonagrioides Lefebvre	628 (183/445/0)	27	10, 11, 16, 20, 26, 29, 33, 36, 41, 42, 46, 49	11, 16, 19, 26, 42, 45	P	BS
27	S. oriaula Tams & Bowden	264 (102/156/6)	25	10, 18, 26, 29	11, 19, 45 ?	P	BS
28	S. pennisetiTams & Bowden	84 (17/66/1)	9	26	11	P	BS
29	S. poephaga Tams & Bowden	248 (194/54/0)	14	1, 8, 14, 18, 19, 26	11, 16, 26, 42, 45	P	BS
30	Sesamia n. sp. 1*	52 (38/14/0)	2	7, 10, 18	11	P	BS
31	Sesamia n. sp. 2*	80 (24/60/0)	1	36	11	P	BS
32	Sesamia n. sp. 3*	415 (294/121/0)	9	9, 10	11, 42, 45	P	BS
33	Sesamia n. sp. 4*	50 (16/34/0)	3	10	16	P	BS
34	Sesamia n. sp. 5*	1215 (1017/186/12)	12	26	16, 26, 42, 45	P	BS
35	Sesamia sp nr coniota	4 (0/4/0)	1	18	16	P	BS
	Speia						
36	Sp. vuteria Stoll	53 (6/40/7)	7	49	11, 19, 45	P	FL

Table 4. Wild host plants of the different stem borer genus in East Africa.
N: reference number, Ac.: *Acrapex*, Bu.: *Busseola*, Ca.: *Carelis*, Busl: *Busseola sensu lato*, Ma.: *Manga*, Po.: *Poeonoma*, Sc.: *Sciomesa*, Se.: *Sesamia*, Sp.: *Speia*, I: individual abundance, S(M): species richness (Number of monophagous species), NG: Number of Genera, α = Fisher-Williams diversity index

N°	Species name	Ac	Bu	Ca	Busl	Ma	Po	Sc	Se	Sp	I	S (M)	NG	α
	Poaceae													
1	*Andropogon amethystinus* Steud								1		3	1	1	0.53
2	*Arundo donax* L.		1								5	1	1	0.30
3	*Cenchrus ciliaris* L.							1			1	1	1	
4	*Cymbopogon nardus* (L.) Rendle		1		1						85	2	2	0.37
5	*Cynodon aethiopicus* Clayton & Harlan		1					1			183	1 (1)	1	0.14
6	*Cynodon dactylon* (L.) Pers.								1		13	1	1	0.25
7	*Cynodon nlemfuensis* Vanderyst		1					1	1		22	3	3	0.94
8	*Digitaria milanjiana* (Rendle) Stapf								1		29	1	1	0.20
9	*Echinochloa haploclada* (Stapf) Stapf							1			12	1	1	0.26
10	*Echinochloa pyramidalis* (Lam.) Hitchc. & Chase		1		1			1	4		653	7 (2)	4	1.10
11	*Eriochloa fatmensis* (Hochst. & Steud.)W.D. Clayton								2		251	2	1	0.30
12	*Eriochloa meyerana* (Nees) Pilg.							1	1		141	2	2	0.33
13	*Euclaena mexicana* Schrader							1			35	1	1	0.19
14	*Hyparrhenia papillides* (A. Rich.) Stapf		1								4	1	1	0.43
15	*Hyperthelia dissoluta* (Steud.) W.D. Clayton								1		3	1	1	0.53
16	*Miscanthus violaceus* (K. Schum.) Pilg.								2		10	2	1	0.75
17	*Panicum deustum* Thunb.		1			1		2			728	4 (2)	3	0.56
18	*Panicum maximum* Jacq.		1		1	2			3		3542	7 (3)	4	1.12
19	*Panicum merkeri* Mez		1						1		85	2	2	0.37
20	*Panicum poaeoides* Stapf								1		16	1	1	0.24
21	*Panicum porphyrhizos* Steud.								1		31	1	1	0.20
22	*Paspalidium geminatum* (Forssk.) Stapf								1		29	1	1	0.20
23	*Pennisetum hohenackeri* Steud.				1			1			178	2	2	0.32
24	*Pennisetum cladestinum* Chiov.						1	1			20	2	1	0.85
25	*Pennisetum macrourum* Trin.		1				1	1			75	3	3	0.38
26	*Pennisetum purpureum* Schumach.		3				1	1	6		3860	11 (3)	4	1.39
27	*Pennisetum trachyphyllum* Pilg.				1			2			808	3 (1)	2	0.40
28	*Pennisetum unisetum* (Nees) Benth.				1						30	1	1	0.20
29	*Phragmites mauritianus* Kunth.							1	3		48	4	2	1.26
30	*Rottboellia cochinchinensis* (Lour.) Clayton							1	1		7	2	2	0.94
31	*Setaria megaphylla* (Steud.) T. Duran & Schinz		1	1	3	1		1			1769	7 (5)	3	0.93
32	*Setaria plicatilis* (Hochst.)Engl.		1			1					186	2	2	0.31
33	*Sorghum arundinaceum* (Desv.) Stapf		2			1			2		241	5	3	0.89
34	*Sporobolus macranthelus* Chiov.	1	1								90	1 (1)	2	0.16
35	*Vossia cuspidata* (Roxb.) Griff.								1		24	1	1	0.21
	Cyperaceae													
36	*Cyperus articulatus* L.							1	3		166	4 (3)	2	1.01
37	*Cyperus atroviridis* C.B. Clarke							2	1		46	3	2	0.98
38	*Cyperus dereilema* Steud			1				1	1		16	3 (1)	2	0.64
39	*Cyperus dichroöstachyus* A. Rich.			1				1			49	2 (1)	2	0.42
40	*Cyperus distans* L.								1		6	1	1	0.34
41	*Cyperus dives* Del.							2	2		118	4	2	0.80
42	*Cyperus exaltatus* Retz.								2		15	2	1	0.62
43	*Cyperus involucratus* Rottb.							1	1		9	2	2	0.80
44	*Cyperus latifolius* Poir.							1	1		4	2	2	1.59
45	*Cyperus maculatus* Boeck.								1		8	1	1	0.30
46	*Cyperus rotundus* L.							1	2		29	3	2	0.84
47	*Schoenoplectus maritimus* (L.)K. Lye							1			3	1	1	0.53
48	*Scleria racemosa* Poir			1							14	1(1)	1	0.25
	Typhaceae													
49	*Typha domingensis* Pers.							1	1	1	618	3 (1)	3	0.41

science. Four species, *Sciomesa mesophaea* (Aurivillius) 1910, *S. piscator* Fletcher 1961, *S. calamistis* and *S. nonagrioides* (Lefebvre) 1827, were found on at least two host plant families and can therefore be considered to be polyphagous (cf Bernays & Chapman 1994). *S. nonagrioides* was found on all three host plant families. Seven species, including *B. fusca*, were oligophagous (feeding on a restricted number of plant species usually from one family or a subfamily (Bernays & Chapman 1994); and were found on two and seven host species within the same family. However, all seven species were more abundant on one or two host plants (i.e., about 85% of the specimens collected). A good example is *M. melanodonta*, of which 88.2 % were collected from *P. maximum* and 10 % from *S. plicatilis* (Hochstetter) Hackel, while the five other hosts together accounted for less than 2%. The host range status of the *B. phaia* Bowden 1956 group is not completed as identification is still in progress. The other 24 species are monophagous (feeding on a single species or genus of plants; Bernays & Chapman 1994).

The found stem borer larvae could be broadly divided into three types, based on colour and adornment: (a) *B. fusca* like species with dark ground colours sometimes adorned with longitudinal wide pale bands (*Busseola*, *Manga* and *Poeonoma*), (b) species with pale ground colours adorned with longitudinal thin dark stripes (*Busseola s.l.* and *Sciomesa* species related to *S. nyei* Fletcher 1961) and (c) *Sesamia* like species with pink ground colours (*Acrapex*, *Carelis*, *Sciomesa* not related to *S. nyei*, *Sesamia* and *Speia*), sometimes adorned with longitudinal wide pale bands [*S. mesophaea*, *S vuteria* (Stoll) 1783]. According to the feeding site, the *Sesamia*, *Acrapex* and *Carelis* species belong to the *Sesamia* feeding type while the *Busseola*, *Busseola s.l.*, *Manga*, *Poeonoma* and *Speia* species belong to the *Busseola* feeding type. Both kinds of feeding behaviour were found in *Sciomesa* genera. A stem girdling behaviour was observed in *S. piscator*. Scarifying behaviour was facultative for both *S. mesophaea* and *S. vuteria* recovered from *Typha domingensis* Persoon.

Discussion

Vegetation mosaics

The results of the surveys indicated that noctuid stem borers are common in the six main vegetation mosaics of East Africa. However, borer species richness and abundance varies amongst the vegetation mosaics with the wet and hot guineo-congolian mosaic covering mainly the western area of East Africa (western Kenya and Uganda) being the richest. Similarly, Nye (1960)

reported noctuid stem borer larvae mainly from the wetter parts of different vegetation mosaics. Nonetheless, in contrast to earlier surveys (Ingram 1958; Nye 1960; Seshu Reddy 1989), higher numbers of stem borer species were collected from non cultivated host plants. Each vegetation mosaic harboured dominant borer species accounting at least for 25% of the specimens collected per mosaic. We found rare species in the five vegetation mosaics. The strong influence exerted by rare species on the overall species assemblage has been underlined many times. However, their role and importance in structuring broad community patterns among regions is not well understood but they are thought to contribute significantly to diversity in the tropics (Price *et al.* 1995; Coddington *et al.* 1996; Novotny & Basset 2000). Not surprisingly the number of rare species was increasing with time and we can assume their contribution to the noctuid borer guild was underestimated in the course of this survey. As all the rare species found are monophagous, no doubt the proportion of specialist feeders was probably also underestimated.

Host plants

Stem borers were recovered from 48 plant species of which 32 have never been recorded as hosts of noctuids, thus increasing the number of known hosts to 66. Most of these hosts belonged to Poaceae family, corroborating earlier reports by Ingram (1958), Nye (1960), Seshu Reddy (1989), and Polaszek & Khan (1998). This study revealed important variation in species richness (S) and diversity index among the host plants. The species diversity reported on some grass species e.g. *Setaria megaphylla* was underestimated because at least three noctuid species collected from it could not be reared to adult stage as all collected larvae died. Data on resource availability suggest strongly that if plants species are extremely abundant and reliable, insects are able to specialize on them and often do so (Bernays & Chapman 1992). It is worth noting that *S. megaphylla* is one of the most common Poaceae species in mid (between 1000 and 1500 m) and high altitude forested areas (≥1500 m). It contained one of the highest borer species diversities (seven) with 71% monophagous species, all of them localised in one or two neighbouring localities usually at altitudes above 1500 m. The current high diversity and monophagy found on *S. megaphylla* could be attributed to major climatic changes of the Pleistocene that took place between 3.3 to 2.45 Million years ago (Wagner 2002; DeMenocal 1995). These major events are considered responsible for the preservation of altitudinal forested areas which worked as refuge zones comparable with an

archipelago (White 1981). The role of these altitudinal forested areas on east Afromontane butterflies speciation and endemism has been well documented by De Jong and Congdon (1993).

Stem borers

The methodology adopted during this survey involved active searching for damage symptoms at irregular intervals in both time and space. Results therefore could not be used for rigorous comparison of stem borer diversity among the different vegetation mosaics, though the methodology allowed for rapid and efficient inventory of the noctuid stem borer guild in East Africa. Out of the 36 species collected, 17 are new to science. The number of known species of noctuids from East Africa is now 50 compare to 32 as reported earlier (Fletcher 1961; Laporte 1975). This study also provided numerous male and female specimens of species earlier described from very few specimens, sometimes from only one sex like *Acrapex syscia* Fletcher 1961, *Sciomesa nyei, Sciomesa venata* Fletcher 1961, *Sciomesa piscator* and *Sesamia oriaula* Tams & Bowden 1953. It is suggested that if the present study is extended to other poorly surveyed areas, the reported diversity of noctuid stem borers from East Africa would increase significantly. The current survey missed the montane biotopes above 2400m and could explain why only one *Acrapex* and 12 *Sesamia* species were recovered even though they are the most diverse noctuid stem borer genera in Africa (Fletcher 1961; Laporte 1975). Stem borer species collected could be categorised into monophagous, oligophagous and polyphagous groups. 63% of monophagous species were found in one vegetation mosaic only, while about 88 % of the oligophagous species were found in at least two vegetation mosaics. The four polyphagous species were present in at least five vegetation mosaics. Nonetheless, the distribution of many monophagous and oligophagous species could not be explained by the distribution of host plants. *Busseola s.l.* n. sp. 2 and *Busseola s.l.* n. sp. 4, that were only known from *S. megaphylla*, were collected from one locality though their host is widely distributed in Guineo-Congolian, afromontane and Zanzibar-Inhambane mosaics. *Sesamia* n. sp. 5 known only from *Pennisetum purpureum* was never recovered in the western part of the Rift Valley despite the wide distribution of the host plant in Kenya and Tanzania. Likewise, *Poeonoma serrata* Hampson 1910 which is widely distributed in Uganda and western Kenya and which was known from *P. purpureum* mainly was never found in the eastern part of the Rift Valley. The observed restricted distribution among stem borer

species may be associated with major climatic changes experienced in East Africa since 3.3 Million years ago, oscillating between hot/humid and cooler/drier periods. These climatic changes coupled with the rise and expansion of the East Africa Rift Valley from 2.9 to 1.6 Million years ago (Zeitoun, 2000, Pitra *et al.*, 2002), were responsible for oscillations in savannah biotype expansion (Wagner 2002; DeMenocal 1995) and of a mosaic of refuge zones.

This study describes mature larval colouration and feeding behaviour of 29 species and six genera for the first time. In addition, it confirms two types of feeding behaviour reported in previous studies (Ingram 1958; Nye 1960). It was found that species belonging to one genus adopt a similar feeding behaviour with a notable exception within the *Sciomesa* genus. All the stem borer larvae with dark ground colours or with dark stripes belong to the *Busseola fusca* feeding type and all the stem borer larvae with pink ground colours belong the *Sesamia calamistis* type. It is not coincidental that larvae living outside the plants develop more pigmented and adorned skin than those ones living inside. When living outside, the larvae are more vulnerable to natural enemies and thus develop camouflage patterns like hiding colour and adornments. Reports indicate that herbivorous species frequently use stripes to escape predators (Caillois 1963; Ortolani 1999).

Conclusion

This paper provides for the first time basic information on noctuid species diversity and biogeography within the six main vegetation mosaics found in East Africa. Even though boring of monocot stems is a very specialized feeding behaviour among noctuids (Holloway, 1998), the major life history traits reported in this study show important behavioural plasticity between genera. Species belonging to the same genus harbour common ecological characteristics like colour of the larvae and feeding behaviour. The *Sciomesa* genus is an exception with a mixture of these characters and further investigations on its systematic and phylogenetic classification might show that it constitutes several genera.

References

Africa AWhere-ACT database 2002. Mud Springs Geographers, Inc. Temple, Texas.

Bernays E.A., Chapman R.F. 1994. *Host-plant selection by phytophagous insects.* Chapman & Hall Inc., New York, 312 p.

Caillois R. 1963. *Le mimétisme animal.* Hachette, 106 p.

Coddington J.A., Young L.H., Coyle F.A. 1996. Estimating spider species richness in a southern Appalachian cove hard-wood forest. *Journal of Arachnology* **24**: 111-128.

De Jong R., Congdon T.C.E. 1993. The montane butterflies of the eastern afrotropics. p. 133-164 *in*: **Lovett J.C., Wasser S.K. (eds.),** *Biogeography and ecology of the rain forests of eastern Africa.* Cambridge University Press, Cambridge.

De Menocal P.B. 1995. Plio-Pleistocene African climate. *Science* **270**: 53-59.

Fletcher D.S. 1961. Noctuidae. Ruwenzori Expedition 1952, **1**: 177-323. British Museum (Natural History), London.

Gounou S., Schulthess F. 2004. Sparial distribution of lepidopterous stemborers on indigenous host plants in West Africa and its implications for sampling schemes. *African Entomology* **12** (2): 171-178.

Holloway J.D. 1998. Noctuidae, p.79-86 *in*: **Polaszek A. (ed)**, *African cereal stem borers : economic importance, taxonomy, natural enemies and control.* CAB International, Wallingford.

Ingram W.R. 1958. The lepidopterous stalk borers associated with graminae in Uganda. *Bulletin of Entomological Research* **49**: 367-383.

Johnson C.D. 1990. Coevolution of Bruchidae and their hosts: evidence, conjecture and conclusions, p. 181-188 *in*: **Fuji K., Gatehouse A.M.R., Johnson C.D., Mitchel R., Yoshida Y. (eds.),** *Bruchids and Legumes: Economics, Ecology and Coevolution.* Kluwer Academics Publishers, New York.

Kergoat G.L., Delobel A., Silvain J.-F. 2004. Phylogeny and host-specificity of European seed beetles (Coleoptera, Bruchidae), new insights from molecular and ecological data. *Molecular Phylogenetics and Evolution* **32**: 855-865.

Laporte B. 1975. Diagnoses de 17 nouvelles espèces de Noctuidae d'Ethiopie et du Kenya (Lépidoptères) (2ème note). *Bulletin mensuel de la Société Linnéenne de Lyon* **44**: 277-287.

Novotny V., Basset Y. 2000. Rare species in communities of tropical insect herbivores: pondering the mystery of singletons. *Oikos* **89**: 564-572.

Nye I.W.R. 1960. The insect pests of graminaceous crops in East Africa. *Colonial Research Studies* **31**: 1-48.

Onyango F.O., Ochieng'-Odero J.P.R. 1994. Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia experimentalis et Applicata* **73**: 139-144.

Ortolani A. 1999. Spots, stripes, tail tips and dark eyes: Predicting the function of carnivore colour patterns using the comparative method. *Biological Journal of the Linnean Society* **67**: 433-476.

Pitra C., Hansen J.A., Lieckfeldt D., Arctander P. 2002. An exceptional case of historical outbreeding in African sable antelope populations. *Molecular Ecology* **11**: 1197-1208.

Polaszek A., Khan Z.R. 1998. Host plants, p.4-10 *in*: **Polaszek A. (ed)**, *African cereal stem borers: economic importance, taxonomy, natural enemies and control.* CAB International, Wallingford.

Price P.W., Diniz I.R., Morais H.C., Marques E.S.A. 1995. The abundance of insect herbivore species in the tropics, the high local richness of rare species. *Biotropica* **27**: 468-478.

Randriamananoro J.J. 1996. *Behavioural and physiological responses of the cereal stalk borer Busseola fusca Fuller (Lepidoptera : Noctuidae) to selected cultivated and wild host plants.* Ph. D. Dissertation, University of Acrra, Ghana, 241 p.

Rougeot P-C. 1984. Missions entomologiques en Ethiopie, 1976-1982. Mémoires du Muséum National d'Histoire Naturelle, nat, (n.s.) *Annale de Zoologie* 1-93.

Samways M.J. 1984. A practical comparison of diversity indices on a series of small agricultural ant communities. *Phytophylactica* **16**: 275-278.

Seshu Reddy K.V. 1989. Sorghum stem borers in eastern Africa, p. 33-40 *in : Proceedings of the International Workshop of sorghum stem borers*, 17-20 November 1987, ICRISAT Centre India. Patancheru, India.

Wagner T. 2002. Late Cretaceous to early Quaternary organic sedimentation in the eastern Equatorial Atlantic. *Palaeogeography, Palaeoclimatology, Palaeoecology* **179**: 113-147.

Walhberg N. 2001. The phylogenetics and biochemistry of host-plant specialization in Melitaeine butterflies (Lepidoptera: Nymphalidae). *Evolution* **55**: 522-537.

White F. 1981. The history of the afromontane archipelago and the scientific need for its conservation. *African Journal of Ecology* **19**: 33-54.

White F. 1983. The vegetation of Africa, a descriptive memoir to accompany the UNESCO / AETFAT / UNSO vegetation map of Africa. *UNESCO, Natural Resources Research* **20**: 1-356.

Zeitoun V. 2000. Adéquation entre changements environnementaux et spéciations humaines au Plio-Pléistocène. *Comptes Rendus de l'Académie des Sciences* **330**: 161-166.

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The role of wild host plants in the abundance of lepidopteran stem borers along altitudinal gradient in Kenya

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Abstract. Presence of wild host plants of stem borers in cereal-growing areas has been considered as reservoirs of lepidopteran stem borers, responsible for attack of crops during the growing season. Surveys to catalogue hosts and borers as well as to assess the abundance of the hosts were carried out during the cropping and non-cropping seasons in different agro-ecological zones along varying altitude gradient in Kenya. A total of 61 stem borer species belonging to families Noctuidae (25), Crambidae (14), Pyralidae (9), Tortricidae (11) and Cossidae (2) were recovered from 42 wild plant species. Two noctuids, *Busseola fusca* (Fuller), *Sesamia calamistis* Hampson, and two crambids, *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* (Strand) were the four main borer species found associated with maize plants. In the wild, *B. fusca* was recovered from a limited number of host plant species and among them were *Sorghum arundinaceum* (Desvaux) Stapf, *Setaria megaphylla* (Steudel) T. Durand & Schinz, *Arundo donax* L. and *Pennisetum purpureum* Schumacher. In contrast, the host range of *C. partellus* was considerably wider [13 for *S. calamistis*]. However, the number of larvae of these species was lower in the wild compared to cultivated fields, thus the role of natural habitat as a reservoir for cereal stem borers requires further studies. Importance of the wild host plants as well as borer diversity along the altitudinal gradient is discussed.

Résumé. Le rôle des plantes hôtes sauvages dans l'abondance des lépidoptères foreurs de graminées selon un gradient altitudinal au Kenya. La présence de plantes hôtes sauvages de foreurs autour des parcelles cultivées a toujours été considérée comme préjudiciable à la production des céréales dans la mesure où elles constituent des réservoirs pour les foreurs. Des enquêtes ont été menées au Kenya, pendant et en dehors des périodes culturales, selon un gradient altitudinal, afin de déterminer le rôle de ces plantes hôtes sur les populations de ravageurs. Soixante et une espèces de lépidoptères foreurs appartenant aux familles des Noctuidae (25), Crambidae (14), Pyralidae (9), Tortricidae (11) et Cossidae (2) ont été récoltées sur 42 plantes hôtes sauvages. Les principales espèces de foreurs associées au maïs sont *Busseola fusca* (Fuller) et *Sesamia calamistis* Hampson (Noctuidae) et *Chilo partellus* (Swinhoe) et *Chilo orichalcociliellus* (Strand) (Crambidae). Dans les habitats sauvages, *B. fusca* a été trouvé sur un nombre restreint de plantes hôtes sauvages telles que *Sorghum arundinaceum* (Desvaux) Stapf, *Setaria megaphylla* (Steudel) T. Durand & Schinz, *Arundo donax* L. and *Pennisetum purpureum* Schumacher. A l'inverse, *S. calamistis* et *C. partellus* ont été trouvées associées à plus de plantes hôtes sauvages [*S. calamistis* (13), *C. partellus* (5)]. Toutefois, le nombre total de chenilles de ces quatre espèces de ravageur trouvé dans les habitats sauvages est très inférieur à celui trouvé dans les parcelles cultivées, aussi le rôle des habitats sauvages en tant que réservoir pour les lépidoptères foreurs de céréales requiert des études plus approfondies. L'importance de la diversité des lépidoptères foreurs dans les plantes hôtes sauvages en fonction du gradient altitudinal est discutée.

Keywords: Stem borer, *Pennisetum purpureum*, *Arundo donax*, Lepidoptera, Africa.

Lepidopteran stem borers are among the most important insect pests infesting maize and sorghum in sub-Saharan Africa (Schulthess *et al.* 1997; Overholt *et al.* 2001; Guofa *et al.* 2002). In East and Southern Africa, *Chilo partellus* (Swinhoe 1884) and *Busseola fusca* (Fuller 1901) are the most important species while *Eldana saccharina* Walker 1865 and *Sesamia calamistis* Hampson 1910 constitute the minor species (Seshu Reddy 1998; Kfir 1997). With the exception of *C. partellus*, which is native to Asia, the other borer species are indigenous to Africa and are assumed to have co-evolved with some native grasses and sedges (Nye 1960; Polaszek & Khan 1998; Overholt *et al.* 2001). Understanding the interactions between these pests and their cultivated and native hosts has been thought of as a prerequisite for developing sustainable management strategies (Bowden 1976).

In East and Southern Africa, populations of Noctuidae and Crambidae often occur as a community of species with overlapping spatial and temporal distribution. In Kenya, *B. fusca* and *C. partellus* are the main pests of maize [*Zea mays* L.] and sorghum [*Sorghum bicolor* (L.) Moench] (Ong'amo 2005). While *B. fusca* dominates the high altitude areas, *C. partellus* is recorded mainly in the lowlands and mid-altitudes (Seshu Reddy 1983; Overholt *et al.* 2001; Ong'amo *et al.* 2006). Crop residues have been reported as responsible for re-establishment of pest populations early in the cropping season (Ingram 1958; Nye 1960). However, information on the role of wild hosts in carry-over of pest populations is scanty.

Stem borers occur in large numbers in maize and sorghum plants during cropping seasons (Songa *et al.* 1998), and their populations survive in wild hosts or in crop stubbles as diapausing larvae during crop free periods (Ingram 1958; Nye 1960; Polaszek & Khan 1998; Haile & Hofsvang 2001). Alternative hosts in the vicinity of the crop fields and crop residues enhance survival of borers during off-season, and thereby are responsible for pest attacks on crops in the subsequent season (Polaszek & Khan 1998). In contrast, oviposition preference studies showed certain wild grasses to be highly attractive to ovipositing moths, though larval survival and adult fecundity are generally low (Haile & Hofsvang 2002). Based on these interactions, hypotheses has been created and validated with field and laboratory trials for *S. calamistis* and *E. Saccharina* (Shanower *et al.* 1993; Schulthess *et al.* 1997). Low borer incidences in maize fields in the forest zones of Cameroon, Ivory Coast and Ghana were partly attributed to abundant wild grasses in the surrounding fields (Schulthess *et al.* 1997). These views appear to differ either because generated hypotheses have not been fully tested or because of differences in borer species. This study was initiated to catalogue hosts and borers in Kenya, and estimates their abundance along different altitudinal gradients.

Materials and methods

Description of the surveyed gradients

Surveys were made during 2003/2004 cropping and non-cropping seasons in 31 localities randomly selected in maize producing areas in Kenya (fig. 1). Localities were grouped in three altitudinal gradients [< 1000, 1000-1500 and >1500 m above sea level (asl)]. Both 1000-1500 and >1500m asl zones are characterized by extensive maize monocultures producing about 80% of the crop consumed in Kenya (De Groote 2002). The other zone (< 1000 asl) is mainly occupied by subsistence farmers who produce approximately 20% of the total maize consumed in the country.

Rainfall in these zones is highly variable and generally bimodal in distribution. This allows for two annual cropping seasons, the first lasting from March - April to May - August (long rainy growing season) and the second from October to December (short rainy season). Most farmers regard long rainy season as the most important season as it is more reliable.

Cultivated host (maize)

Ovipositing noctuids are reported to have a strong preference for pre-tasseling crops and thus attack plants early in the season. There is one or two stem borer generations during the short rainy season depending on the crop cycle and the duration of pre-tasseling unlike during the long rainy season where there are two generations. Long rainy season populations are thought to either come from adjacent earlier planted crops or wild habitats. This study thus aimed at capturing the first generation of the long rainy season. Several maize farms within a radius of 400m of each survey locality were sampled. Visits were made during four to six weeks after germination of maize during the long rainy season. In each session, 100 randomly selected maize stems were inspected for stem borer infestation. Infested stems were cut and dissected for larval and pupal recovery. Other cultivated graminaceous crop-hosts such as *S. bicolor*, *Saccharum officinarum* L., and *Eleusine corocana* L. were also sampled. However these hosts were sampled in very few localities and are presented together with the wild host plants (tab. 2).

Wild host plants

Surveys in wild host habitats in the vicinity of crop fields were done during both cropping and non-cropping seasons. Since borer densities on wild hosts plants are considerably lower than on cultivated cereals (Nye 1960; Schulthess *et al.* 1997), selective sampling was adopted to increase the chances of finding borers. In each locality, all potential hosts belonging to the Poaceae, Cyperaceae and Typhaceae families found within 200-300 m from the border of cultivated maize field were carefully examined for symptoms of stem borer damage. Damaged plants were cut at the bases and dissected for recovery of larvae and pupae. Plants from which the larvae or pupae were collected were identified. In cases where identities of the infested plants were not known, voucher specimens were taken to the East African herbarium in Nairobi for identification.
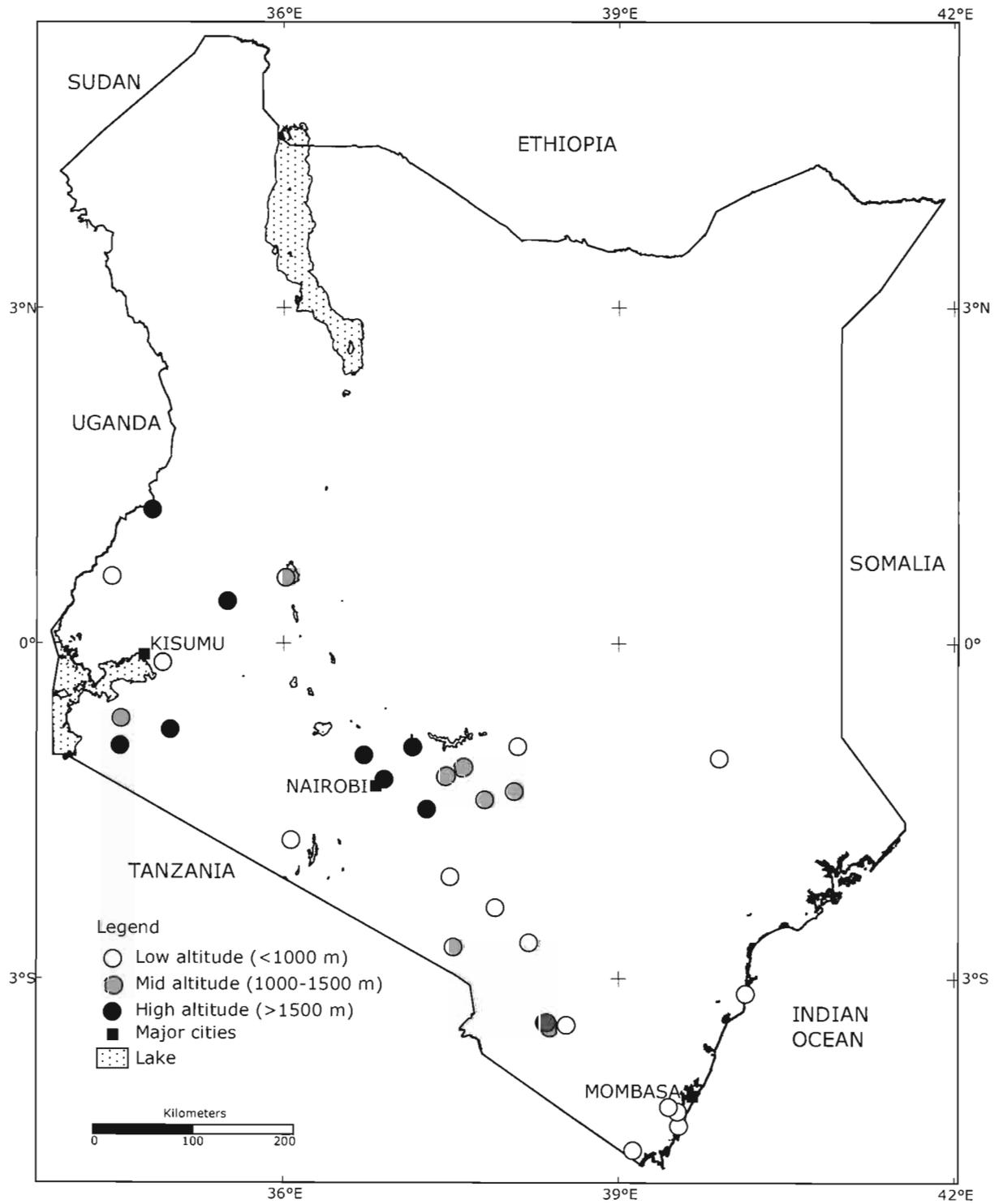
Figure 1
Map of Kenya outlining localities surveyed for stem borer infestation in both maize and wild hosts during the study.

Stem borer species

Upon dissection, recovered larvae were individually introduced into glass vials containing *B. fusca* diet (Onyango & Ochieng'Odero 1994) where they remained until pupation or emergence of natural enemies. Pupae were then individually transferred to separate plastic vials until adult emergence. Noctuid moths were shipped to Centre National de la Recherche Scientifique, Gif-sur-Yvette Cedex (France) where they were identified by one of us (Pascal Moyal). Apart from *C. partellus* and *E. saccharina*, identity of the crambids, pyralids, tortricids and cossids could not be confirmed and are thus presented in their tentative families. Specific characters used for identification of *Chilo* species appear to be very variable (Blezynski 1970) and more so for species close *Chilo orichalcociliellus* (Strand, 1911). To avoid risk of misidentification, materials close to *C. orichalcociliellus* are presented here as *C. orichalcociliellus* group. In addition, presentations of some materials in the text are limited to genera (Noctuidae family) and super-families (Pyraloidea, Tortricoidea and Cossoidea).

A correlation test was performed to estimate the relationship between the relative importance of stem borer species and altitude.

Results

Stem borer abundance in maize fields along the altitude

Busseola sp nr *phaia*, *Sciomesa piscator* Fletcher 1961, *Sesamia nonagrioides botanephaga* (Lefebvre 1827) and *Sesamia* sp nov 5 were found infesting maize in addition to *B. fusca*, *C. partellus*, *S. calamistis* and *C. orichalcociliellus* group which were the main pest species (tab 1). Pest species coexisted in most localities with varying densities along the altitudinal gradient ($F_{2,171}$ = 8.86; p = 0.0002). The highest density (1.39 larvae/plant) was recorded in mid altitude gradient followed by low altitude gradient

Table 1. Stem borer pests collected among maize plants in different localities along the altitudinal gradient.
In parenthesis along columns are the number of larvae collected among wild host plants; *B.f.* - *B. fusca*; *S.c.*- *S. calamistis*; *C.p.* - *C. partellus*; *C.o.* group - *C. orichalcociliellus* group.

	Localities	Latitude	Longitude	Altitude (m asl)	Larvae from cultivated crops			
					B. f.	*S. c.*	*C. p.*	*C. o.* group
Low altitude (<1000)	Malindi	S 03°08.054'	E 40°08.098'	33	0	112	774(2)	22(12)
	Mombasa 6	S 04°19.196'	E 39°32.471'	43	0	36(32)	295(30)	16(120)
	Mombasa 8	S 04°32.502'	E 39°07.831'	103	0	84(8)	676(6)	4(5)
	Shimba 1	S 04°11.463'	E 39°31.921'	111	1	76	287	75(9)
	Bura	S 01°02.007'	E 39°53.988'	148	0	9	357(36)	0
	Shimba 2	S 04°08.843'	E 39°26.963'	417	0	53	456(2)	83(96)
	Taita 3	S 03°24.917'	E 38°32.075'	610	0	1	976(210)	0
	Mombasa 7	S 02°40.627'	E 38°11.715'	739	10	81	487(201)	0(82)
	Rift Valley 3	S 01°45.837'	E 36°03.991'	914	4	17(3)	98(275)	0
	Mombasa 3	S 02°21.894'	E 37°53.528'	989	20	19(3)	387(78)	0(2)
	Garissa 2	S 00°55.672'	E 38°05.676'	994	2	13(3)	520(19)	0(379)
Mid altitude (1000 – 1500)	Rift Valley 6	N 00°35.168'	E 36°00.921'	1084	22	13(100)	0	0
	Kisumu 2	S 00°10.357'	E 34°54.804'	1143	128	16	1014	0
	Mombasa 2	S 02°05.453'	E 37°29.388'	1153	19	29	1400(372)	0
	Kitui1	S 01°24.114'	E 37°48.047'	1160	2	7	384	0
	Mt. Kenya 2	S 00°71.720'	E 37°26.730'	1179	88	66(23)	1726(494)	1
	Taita 2	S 03°26.292'	E 38°21.955'	1180	81	15	114(4)	0
	Homa Bay 1	S 00°40.382'	E 34°32.128'	1250	101	9	580	0
	Kisumu 4	N 00°35.775'	E 34°27.165'	1283	227	20	484	0
	Loitoktok	S 02°43.109'	E 37°31.169'	1331	20	68(2)	278(12)	0
	Kitui 2	S 01°19.482'	E 38°03.684'	1363	106(2)	47(31)	166	0
	Garissa 1	S 01°07.185'	E 37°35.879'	1363	2	65	833	0
High altitude (>1500)	Kakamega	N 00°22.530'	E 34°89.660'	1551	209(87)	3(16)	0	0
	Kisii 2	S 00°54.790'	E 34°31.740'	1583	60	1(9)	16	0
	ICIPE	S 01°13.209'	E 36°53.775'	1625	129	26(4)	41	0
	Mt. Kenya1	S 00°55.793'	E 37°09.343'	1639	595	92	639(2)	0
	Taita 1	S 03°23.626'	E 38°20.339'	1729	618	90	1	0
	Machakos	S 01°29.347'	E 37°16.611'	1978	707	7	0	0
	Kitale 2	N 01°11.738'	E 34°49.106'	2160	603(47)	1(7)	2	0
	Gatamaiyu	S 01°00.057'	E 36°43.022'	2181	297(2)	7	0	0
	Kisii 1	S 00°46.216'	E 34°58.788'	2223	279	3(9)	0	0

(0.84 larvae/plant), while the lowest density (0.80 larvae/plant) was recorded in high altitude gradient.

Stem borer community significantly varied along the altitudinal gradient ($F_{11,112}$ = 54.62; p < 0.0001). Distribution of *B. fusca* was strongly correlated to the increase in altitude ($F_{1,29}$ = 25.65; p < 0.001; r^2 = 0.42)

with more larvae in high altitude zone (>1500 m) where it constituted about 85% of the community. A negative relationship was observed in the proportion of both *S. calamistis* ($F_{1,29}$ = 4.80; p < 0.05; r^2 = 0.14) and *C. orichalcociliellus* group ($F_{1,29}$ = 8.80; p < 0.05; r^2 = 0.23) with an increase in altitude though they constituted

Table 2. Host plants from which different stem borer super-families were recovered.

Family	Host plants	Noctuoidea	Pyraloidea	Tortricoidea	Cossoidea
Poaceae	*Arundo donax* L.	6	2	0	0
	Cymbopogon nardus (L.) Rendle	25	0	0	0
	Cynodon aethiopicus Clayton & Harlan	103	0	0	0
	Cynodon dactylon (L.) Persoon	13	0	0	0
	Cynodon nlemfuensis Vanderyst var. *nlemfuensis*	55	0	0	0
	Digitaria milanjiana (Rendle) Stapf	28	5	2	0
	Echinochloa pyramidalis (Lam.) Hitchc. & Chase	178	141	0	7
	Eleusine corocana L.	40	0	0	0
	Eriochloa fatmensis (Hochsttetter & Steudel) Clayt.	176	0	0	0
	Eriochloa meyerana (Nees) Pilger	64	2	0	0
	Euclaena mexicana Schrader	0	14	0	0
	Hyparrhenia papillides (Hochstetter) Stapf	4	4	0	0
	Hyperthelia dissoluta (Steudel) Clayton	4	4	0	0
	Panicum deustum Thunb	55	22	0	0
	Panicum maximum Jacquin	1189	547	0	0
	Panicum merkeri Mez	37	16	0	0
	Panicum porphyrhizos Steudel	31	0	0	0
	Paspalidium geminatum (Forskal) Stapf	29	0	0	0
	Pennisetum macrourum Trinius	41	0	0	0
	Pennisetum purpureum Schumacher	1569	146	0	0
	Pennisetum trachyphyllum Pilger	491	0	0	0
	Phragmites mauritianus Kunth	3	1	0	3
	Rottboellia cochinchinensis (Loureiro) Clayton	6	201	0	0
	Saccharum officinarum L.	49	0	0	0
	Schoenoplectus corymbosus (Roemer & Schultes)	0	0	13	0
	Setaria megaphylla (Steudel) T. Durand. & Schinz	742	0	0	0
	Sorghum arundinaceum (Desvaux) Stapf	162	1761	0	0
	Sorghum bicolor (L.) Moench	539	2330	0	0
	Vossia cuspidata (Roxburg) Griffith	24	0	0	0
	Zea mays L.	5862	13192	0	0
Cyperaceae	*Carex chlorosaccus* C.B. Clarke	0	14	0	0
	Cyperus latifolius Poiret	7	21	0	0
	Cyperus maculatus Boeck.	0	0	35	0
	Cyperus alopecuroides Rottboll	0	6	20	0
	Cyperus articulatus L.	12	0	55	0
	Cyperus dereilema Steudel	0	14	0	0
	Cyperus dichrostachyus A. Richard	19	37	0	0
	Cyperus distans L.	25	18	14	0
	Cyperus dives Delile	77	18	10	0
	Cyperus exaltatus Retzius	5	29	7	0
	Cyperus prolifer Lamark	0	0	24	0
Typhaceae	*Typha domingensis* Persoon	207	38	0	0

Very low number of larvea were recovered from *Setaria sphacelata* (Schumacher) Moss, *Echinochloa haploclada* (Stapf) Stapf, *Cenchrus ciliaris* L., *Cyperus rotundus* L. and *Cyperus involucratus* Rottboll and have thus been excluded from the table.

the minor proportion of the borer community in low altitude zone (8 and 3% respectively). Unlike the other species, frequencies of *C. partellus* did not vary with increase in altitude ($F_{1,29}$ = 3.92; p > 0.05; r^2 = 0.12) though it dominated mid- and low altitude zones where it constituted 72 and 86% respectively.

Stem borer pests among wild host plants

Stem borer species recovered from maize plants were also obtained from wild host plants growing in the vicinity of crop fields. Their abundance varied among localities along the altitudinal gradient. *B. fusca* was recovered only from five localities on four Poaceae species, namely *Setaria megaphylla* (Steudel) T. Durand & Schinz (Kakamega) *Arundo donax* L. (Mt. Kenya 1), *Sorghum arundinaceum* (Desvaux) Stapf. (Kakamega, Kitui 2 and Kitale 2) and *Pennisetum purpureum* Schumacher (Gatamaiyu). These localities were situated in both the mid- (Kakamega and Kitui 2) and high altitude (Mt. Kenya 1, Kitale 2 and Gatamaiyu) zones. In contrast, *S. calamistis* was recovered in 14 localities from 13 different host plants belonging to Poaceae (9) and Cyperaceae (4) families (tab. 2). *S. calamistis* larvae were recovered mainly from *Cyperus distans* L., *Eleusine corocana* L., *Panicum porphyrhyzos* Steudel, *S. arundinaceum*, *Paspalidium geminatum* (Forskol) Stapf and *Vossia cuspidata* (Roxburg) Griffith. However, *S. arundinaceum* was found infested in many localities (Kitale 2, Mombasa 3, Mombasa 6, Mt. Kenya 2 and Rift valley 3) across all altitudinal gradients.

Like in maize, crambids *C. partellus* and *C. orichalcociliellus* group occurred mainly in low altitude localities. *C. partellus* was mainly collected from *S. arundinaceum*, *P. purpureum*, *Rottboellia cochinchinensis*

(Loureiro) Clayton and *Panicum maximum* Jacquin. However, *S. arundinaceum* was frequently found infested by this species in both low and mid altitude localities. On the other hand, the *C. orichalcociliellus* group was found restricted to low altitude zone where it was recovered from seven different plants: *S. arundinaceum*, *P. purpureum*, *P. maximum*, *Digitaria milanjiana* (Rendle) Stapf, *Euclaena mexicana* Schrader, *P. deustum* Thunberg and *Hyperthelia dissoluta* (Steudel) W.D. Clayton. *P. maximum* was frequently found infested by this species in five localities. In some localities (Mombasa 3, Mombasa 6, Mombasa 7, Shimba 2 and Garissa 2), *C. orichalcociliellus* group immatures were more frequent on wild hosts compared to maize. *E. saccharina* was recovered from two localities on two Cyperaceae species namely *Cyperus dives* Delile and *Cyperus alopecuroides* Rottboll.

Non-pest stem borers among wild host plants

Stem borers varied in their distribution in wild host plants among different zones without any consistent pattern (tabs. 2 & 3). Some localities, particularly in the high altitude zone (Kakamega, Gatamaiyu, Kisii 1 and Kisii 2) had more larvae of "non-pest species" on wild hosts compared to maize (tab. 2). Similar results were recorded in the low altitude localities namely Mombasa 6, Mombasa 7, Rift Valley 3 and Garissa 2. About 22 non-pest borer species belonging to 7 different genera within the noctuid family have been identified. These genera varied in terms of species richness among which *Sesamia* Guenée 1852 had 9 species followed by *Sciomesa* Tams and Bowden 1953 (6), *Manga* Bowden 1956 (2) and *Busseola* Thurau 1904 (1). Three unknown species were also recovered

Table 3. Proportions (%) of stem borer genera and families in different altitudinal zones from wild host plants. Parenthesis along the genera column indicates the number of species.

Super-family	Family	Genera	<1000	1000–1500	> 1500
Noctuoidea	Noctuidae	*Busseola* (1)	1.5	0.1	14.7
		Carelis (1)	0	0.0	4.8
		Busseola sensu lato (3)	0	0.0	13.3
		Manga (2)	23.4	9.7	1.9
		Poeonoma (1)	0	0.0	15.8
		Sciomesa (6)	4.6	0.8	38.8
		Sesamia (9)	15.8	28.7	5.8
Pyraloidea	Crambidae	*Chilo* (5)	43.4	56.8	0.1
		Other crambid species (7)	1.8	0.5	3.1
	Pyralidae	*Eldana* (1)	0.1	0.1	0.3
		Other pyralid species (8)	6.9	0.3	0.3
Tortricoidea	Tortricidae	11 species	2.5	2.6	1.1
Cossoidea	Cossidae	2 species	0	0.4	0

and grouped within the unknown genera tentatively named *Busseola* sensu lato. The other genera, *Poeonoma* Tams and Bowden 1953 and *Carelis* Bowden 1956 respectively have one species each (tab. 3). Nonetheless, there was variation in distribution proportion among the genera along altitudinal gradient. The *Manga* genus dominated low altitude localities (< 1000m asl) where it constituted about 51% of noctuid larvae collected, followed by *Sesamia* (34%). *Sesamia* was the most important genus in the mid altitude zone, constituting about 72% of total noctuids collected, while *Sciomesa* was the most abundant genus in high altitude zones constituting about 40% of the total noctuids, followed by the genus *Poeonoma* with 16%.

Twenty-one species belonging to Crambidae and Pyralidae families were recovered (tab. 3). The most abundant among these was the Crambidae family where 2780 larvae belonging to 12 different species were identified. Important in this family was the *Chilo* genus, which had 5 species. Nine species were identified within the Pyralidae family of which *E. saccharina* constituted about 5% of the total collection. Tortricidae was second to Pyralidae in terms of species richness, and third in terms of the number of larvae collected. About 11 species were tentatively identified within Tortricidae from 180 specimens collected. The least number of larvae (10) as well as species (2) were collected from the Cossidae family.

Discussion

This findings support earlier reports on variations in the distribution of *Busseola fusca* and *Chilo partellus* according to altitude (Seshu Reddy 1983; Songa et al. 1998; Guofa et al. 2002). The difference Nye (1960) ascribed to climatic variations especially the temperature. However, pest populations were higher in maize fields compared to wild habitats. High pest occurrence in maize fields indicates better suitability of cultivated crops to support borer populations compared to wild grasses. Shanower et al. (1993) showed that survival of *Sesamia calamistis* and *Eldana saccharina* larvae was less than 10% in *Panicum maximum*, *Sorghum arundinaceum*, *Pennisetum purpureum* and *Pennisetum polystachion* (L.) Schultes, while larval survival in maize was between 19 and 30%. Low borer survival was thus ascribed to poor host quality.

Busseola fusca was found in maize in all localities in the mid and high altitude zones, but it was recovered from four wild host species in five localities only. Contrary to earlier reports (Polaszek & Khan 1998; Overholt et al. 2001), only two larvae of *B. fusca* were recovered from *P. purpureum*, which was found growing within a maize field. Laboratory studies demonstrated that *B. fusca* larvae were unwilling to bore in *P. purpureum* stems and that adult moths would not oviposit on that host plant (Wilkinson 1936, Calatayud et al. in lit.). Very likely reports of *B. fusca* on *P. purpureum* in Kenya may have been a result of the larvae moving from maize or sorghum onto *P. purpureum*, or misidentification. However, *B. fusca* was reported to be common on *P. purpureum* in Central Africa (Cameroon) (Ndemah et al. 2001a) and there may be agreement that host range of most insects is dynamic and often location and time-specific (Polaszek & Khan 1998). Unlike *B. fusca*, *S. calamistis* was recovered from many plant species confirming its polyphagy corroborating reports from West and Central Africa (Ndemah et al. 2001b). Though wild plants are attractive to ovipositing moths, larval survival and adult fecundity are generally low (Shanower et al. 1993), which may explain the low populations observed in maize fields surrounded by wild hosts in Benin and Cameroon (Schulthess et al. 1997; Schulthess et al. 2001; Ndemah et al. 2002).

Chilo partellus was found restricted to low and mid-altitude zones with high populations in maize and low population in the wild habitats. *C. partellus* populations were higher than that of other borers supporting earlier studies which suggested that this zone is ecologically suitable for its establishment. Suitable climatic conditions coupled with available alternative hosts are thought to have favoured *C. partellus* (Guofa et al. 2002). This can explain its rapid population build up that resulted in the displacement of indigenous *Chilo orichalcociliellus* (Seshu Reddy 1983). However, there was evidence of variation in niche occupation among these two species in the wild as most of the *C. partellus* larvae were found on *S. arundinaceum* while the *C. orichalcociliellus* group larvae were found mainly on *P. maximum*.

Recorded diversity is higher than previously reported. However, the distribution of some wild stem borers was found restricted to either a certain plant species or altitudinal zones. The *Manga* genus was commonly found on *P. maximum* in low altitude zones, while *Busseola* spp were found mainly in the high altitudes. Even with the observed variation in distribution with altitude, importance of wild stem borers as alternative hosts of natural enemies during the crop period cannot be ignored. Bonhof (2000) reported high diversity and abundance of natural enemies in maize fields at the Kenyan coast during the beginning of long rainy season. She ascribed this diversity to possible movement of natural enemies from wild habitats where they attack alternative host insects. Schulthess et al. (2001) reported relatively high parasitism of *S. calamistis* eggs by *Telenomous* spp (Hymenoptera: Scelionidae) during the dry season in the Inland valley in Benin and Cameroon. Wild habitats rich in alternative stem borers may attract

and sustain populations of natural enemies that would eventually move to cultivated fields and suppress pest populations.

The presence and abundance of the "wild" stem borer species in different regions appear to be affected by the availability of suitable host plants. Borer species belonging to *Sesamia, Sciomesa, Busseola* genera as well as Crambids, Pyralids and Tortricids constituted an important proportion of the total collection made. Most of the wild stem borer species were recovered from limited number of hosts. According to Hermsmeier *et al.* (2001), such specialist species are more likely to adapt to the toxic compounds they encounter. Distribution and abundance of some wild stem borer species may thus be attributed to their adaptations to overcome plant defences. However, some of the wild borer species (*Busseola phaia* Bowden, 1956 and *Sciomesa piscator*) develop easily on maize stems exhibiting a potential to shift and become pests of cultivated cereals. The recent host switch of *E. saccharina* from sedges to sugar cane in South Africa where it became the key pest (Atkinson 1980) confirms this assumption. *E. saccharina* was only found on wild plants during this study though it is an important pest of maize in West Africa (Bosque-Perez & Mareck 1990). Nonetheless, it could be a major threat to maize crops in Kenya if it eventually shifts to cultivated fields.

Reference

Atkinson P.R. 1980. On the biology, distribution and natural host-plants of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Journal of the Entomological Society of Southern Africa* 43: 171-194.

Blezynski S. 1970. A revison of the world species of *Chilo* Zincken (Lepidoptera: Pyralidae). *Bulletin of the British Museum (Natural History) Entomology* 25: 101-195.

Bonhof M.J. 2000. *The impact of predators on maize stem borers in Coastal Kenya.* PhD thesis, Wageningen University, The Netherlands.

Bosque-Perez N.A., Mareck J.H. 1990. Distribution and species composition of lepidopterous maize borers in southern Nigeria. *Bulletin of Entomological Research* 80: 363-368

Bowden J. 1976. Stem borer ecology and strategy for control. *Annals of Applied Biology* 84: 107-111.

De Groote H. 2002. Maize yield losses from stemborers in Kenya. *Insect Science and its Application* 22: 89-96.

Guofa Z., Overholt W.A., Mochiah M.B. 2002. Changes in the distribution of lepidopteran maize stem borers in Kenya from 1950's to 1990's. *Insect Science and its Application* 21: 395-402.

Haile A., Hofsvang T. 2001. Survey of lepidopterous stem borer pests of sorghum, maize and pearl millet in Eritrea. *Crop protection* 20: 151-157.

Haile A., Hofsvang T. 2002. Host plant preference of the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Crop protection* 21: 227-233.

Hermsmeier D., Schittko U., Baldwin I.T. 2001. Molecular Interactions between the Specialist Herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and Its Natural Host *Nicotiana attenuata*. I. Large-Scale Changes in the Accumulation of Growth- and Defense-Related Plant mRNAs. *Plant Physiology* 125: 683-700.

Ingram W.R. 1958. The lepidopterous stalk borers associated with graminae in Uganda. *Bulletin of Entomological Research* 49: 367-383.

Kfir R. 1997. Competitive displacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America* 90: 619-624.

Ndemah R., Schulthess F., Poehling H.-M., Borgemeister C. 2001a. Natural enemies of lepidopterous borers on maize and elephant grass in the forest zone of Cameroon with special reference to *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research*. 91: 205-212.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Cardwell K.F. 2001b. Distribution, relative importance and effect of lepidopterous borers on maize yields in the Forest zone and Mid-Altitudes of Cameroon. *Journal of Economic Entomology* 94: 1434-1444.

Ndemah R., Gounou S., Schulthess F. 2002. The role of wild grasses in the management of lepidopterous cereal stemborers on maize in western Africa. *Bulletin of Entomological Research* 92: 507-519.

Nye I.W.B. 1960. *The Insect Pests of Graminaceous Crops in East Africa*. Colonial Research Study No. 31, Her Majesty's Stationery Office, London, 48 p.

Ong'amo G.O. 2005. *An assessment of lepidopteran stem borer species distribution in relation to diversity of wild host grasses in maize and sorghum growing zones in Kenya*. MSc thesis, Kenyatta University, Nairobi, Kenya.

Ong'amo G.O., Le Ru B.P., Dupas S., Moyal P., Calatayud P.-A., Silvain J.-F. 2006. Distribution, pest status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. *Annales de la Société Entomologique de France* 42(2): 171-177.

Onyango F.O., Ochieng' Odero J.P.R. 1994. Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia experimentalis et Applicata* 73: 139-144.

Overholt W.A., Maes K.V.N., Goebel F.R. 2001. *Field guide to stem borer larvae of maize, sorghum and sugarcane in Eastern and Southern Africa*. ICIPE Science Press, Nairobi, 31 p.

Polaszek A., Khan Z.R. 1998. Host Plants, p. 3-10 *in*: Polaszek A. (ed.), *African Cereal Stem Borers: Economic importance, Taxonomy, Natural enemies and Control*. CAB International, University Press, Cambridge.

Schulthess F., Bosque-Perez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Goergen G. 1997. Exchange of Natural enemies of lepidopteran cereal stem borers between African regions. *Insect Science and its Application* 17: 97-108.

Schulthess F., Chabi-Olaye A., Georgen, G. 2001. Seasonal fluctuations of noctuid stem borer egg parasitism in Southern Benin with special reference to *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Telenomus* spp. (Hymenoptera: Scelionidae) on maize. *Biocontrol Science and Technology* 11: 754-757.

Seshu Reddy K.V. 1983. Study of the stem borer complex of sorghum in Kenya. *Insect Science and its Application* 4: 3-10.

Seshu Reddy K.V. 1998. Maize and sorghum: East Africa, p. 25-27 *in*: A. Polaszek (Ed), *Africa cereal stemborers: Economic importance, Taxonomy, Natural Enemies and Control*. CAB International. University Press, Cambridge.

Shanower T.G., Schulthess F., Bosque-Perez N.A. 1993. The effects of larval diet on the growth and development on *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana Saccharina* Walker (Lepidoptera: Pyralidae). *Insect Science and its Application* 14: 681-685.

Songa J.M., Overholt W.A., Mueke J.M., Okello R.O. 1998. *Distribution of stem borer species in Semi-Arid Eastern Kenya*, Sixth Eastern and Southern Africa Regional maize conference, 21st - 25th September 1998, p. 117-120.

Wilkinson H. 1936. *Report of the Entomological section*. Representative, 2, Department of Agriculture, Kenya 1935, p. 60-70.

Diversity and abundance of wild host plants of lepidopteran stem borers in two different agroecological zones of Kenya

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Abstract. A survey was carried out between 2004 and 2005 in two ecologically different locations, Kakamega and Muhaka to assess diversity and abundance of wild host plants of lepidopteran stem borers as compared to maize plots during the cropping and non-cropping seasons. Kakamega in Western Kenya is characterized by a Guineo-Congolian rain forest mosaic and Muhaka at the Kenyan coast by a Zanzibar Inhambane mosaic with secondary grassy and woody vegetation. In Kakamega, wild host plants and maize covered 2 and 43% of the surveyed area. No variation in diversity and relative abundance of wild host plants was observed between both the cropping and non-cropping seasons. In Muhaka, the diversity and relative abundance of wild host plant species differed between seasons, with the Shannon Weaver Index (H) of 1.67 and 0.95 for cropping and non-cropping seasons, respectively. Similarly in this location, wild host plant cover varied between cropping (23%) and non-cropping (17.9%). During both seasons, this was higher than the maize cover, with 10.7% and 0% for the cropping and non-cropping seasons, respectively. For both localities, the implication of the differences found in the abundance and diversity between the cropping and non-cropping seasons is discussed.

Résumé. Diversité et abondance des plantes hôtes sauvages des lépidoptères foreurs de graminées dans deux localités écologiquement différentes du Kenya. Une étude a été menée en 2004 et 2005 dans deux localités écologiquement différentes du Kenya, Kakamega à l'Ouest et Muhaka sur la côte afin d'estimer la diversité et l'abondance des plantes hôtes sauvages des lépidoptères foreurs de céréales pendant et en dehors de la saison culturale. Kakamega est caractérisé par une mosaïque de type forêt pluviale Guinéo-Congolaise et Muhaka par une mosaïque de type Zanzibar-Inhambane avec des formations secondaires herbeuses et arborées. A Kakamega, les plantes hôtes sauvages et le maïs occupent respectivement 2 et 43% de la surface étudiée ; la diversité et l'abondance relative des plantes hôtes sauvages ne varient pas avec la saison. Par contre, à Muhaka, la diversité et l'abondance des plantes hôtes sauvages varient selon la saison, l'indice de diversité de Shannon Weaver (H) est de 1,67 et 0,95 respectivement pendant et hors saison culturale. De même, dans cette localité, la surface occupée par les plantes hôtes sauvages varie entre la saison culturale (23%) et hors saison culturale (17,9%) mais reste supérieure à la surface plantée en maïs qui est respectivement de 10,7 et 0%. Pour les deux localités, les implications des différences observées dans l'abondance et la diversité des plantes hôtes sauvages pendant et hors saison culturale sont discutées.

Keywords: Stem Borers, Wild Hosts, Monocotyledons, cropping season, East Africa.

In East Africa, *Busseola fusca* (Fuller, 1901) (Noctuidae) and *Chilo partellus* (Swinhoe, 1884) (Crambidae) are the most important insect pests in the crop fields (Seshu Reddy 1983; Guofa *et al.* 2002). *B. fusca* dominates high altitude areas whereas *C. partellus* is well established in low and mid altitude areas (Seshu Reddy, 1983). Currently, it is assumed that the original hosts of cereal stem borers were wild grasses and sedges, and that the pest species have maintained close association with the wild habitat. In East Africa, the bulk of maize, *Zea mays* L. (Poaceae) and sorghum (*Sorghum bicolor* L. (Moench) (Poaceae) is mainly grown on small plots surrounded by land occupied by vegetation of which the majority are wild host plants of lepidopteran stem borers (Khan *et al.* 1997). During the intercropping period, it is thought that the presence of wild hosts near crop fields favours the survival of stem borers thereby increasing population that colonises crops in subsequent growing season (Ingram 1958; Nye 1960; Seshu Reddy 1989; Randriamananoro 1996; Polaszek & Khan 1998; Haile & Hofsvang 2001). Recent studies done on wild host range of stem borers in East Africa by Le Rü *et al.* (2006a, 2006b) indicate that *B. fusca* and *C. partellus* are oligophagous, contradicting previous report by Polaszek & Khan (1998) characterising these species as polyphagous. Studies done by Kanya *et al.* (2005) in Kitale in Kenya showed that area covered by wild host plants of these pests was below 10% suggesting that wild host plants might not be adequate as refuge of these pests. Until now, little attention has been given to the role of wild host plants in the invasion of crop fields by the stem borer pests. Bowden (1954) argued that the ecology of these pests could only be understood within the context of the wild habitat.

In an attempt to understand the role of wild host plants on pest population dynamics between natural and cultivated habitats, two representative locations, Kakamega and Muhaka, from different agro-ecological zones in Kenya were chosen for their diversity of habitats and farm management practices. Kakamega in Western Kenya is found in moist mid-altitude and is characterized by a Guineo-Congolian rain forest mosaic. Muhaka at the Kenyan coast is found in moist low tropics and is characterized by a Zanzibar Inhambane mosaic with secondary grassy and woody vegetation (Kokwaro 1988). Kakamega is dominated by *B. fusca* while Muhaka by *C. partellus* (Seshu Reddy 1983, Ong'amo 2005). This study was carried out across the natural habitat between the cropping and non-cropping season with a focus on the diversity and abundance of wild host plants of stem borers in the different vegetation associations. This information

could be used to assess the possible survival of stem borer pests on wild habitats during the intercropping period and subsequent outbreak or invasion on cereal crops during the cropping season. It would also provide possible explanation whether wild habitat could delay development of resistance of the stem borer main pests to *Bt*-Maize.

Material and Methods

Study localities and sampling design

The Kakamega locality (fig. 1) covers an area of 21.2 km² and is located 50 km North of Lake Victoria on the border of transitional rain forest in a depression at the bottom of the Nandi escarpment (Kokwaro 1988). Vegetation species within the forest are typical of planetary Guineo-Congolian rain forests. Parts of the forest (study locality) have been opened to cultivation of maize and sorghum because of the favourable climatic conditions (temperature ranges from 12.7 to 27.1 °C and average rainfall is 1650 mm). The altitude ranges from 1551 to 1730 m above sea level (asl). The location is characterized by a bimodal rainfall distribution that allows two cropping season (CS), the first lasting from March to mid-July and the second from mid-August to November. There is a prolonged dry season from December to the end of February (Kokwaro, 1988) herewith referred to as non-cropping season (NCS).

The Muhaka locality (fig. 1), covering an area of 19.3 km², is located on the south of Mombasa in an area with secondary grassy and woody vegetation, on the border of an undifferentiated forest with climatic condition of Inhambane type (minimum temperature, 22 °C; maximum, 30.4 °C; average rainfall, 1212 mm). The altitude ranges from 20 to 67 m above sea level. There is only one cropping season from March to June, the rest of the year too dry spell to grow crops. The locality is characterized by scattered patches of cultivated maize fields (Kokwaro 1988).

In Kakamega, sampling was done in November 2004 for the CS and March 2005 for NCS. In Muhaka, the sampling was carried out in October 2004 for the NCS and in May, 2005 for the CS. The sampling period lasted for three weeks in each session.

Sampling size

High resolution satellite maps of the two locations were used as a basic spatial information to analyse vegetation mosaics in the two locations. Ground actualization was carried to further describe the vegetation formations. The resultant land use map characterized the locations into various homogeneous vegetation structures containing natural and cultivated habitats of stem borers. Sampling was done in the natural habitats inhabited by wild hosts within the vegetation structures described by the satellite land use map of the two locations (Guiheneuf 2004). In Kakamega, four vegetation structures made up of uncultivated habitats were identified while Muhaka had five (tab 1). Geographical Information System (GIS) program, Arc View version 3.2 (ESRI 1992) software was used to generate random sampling points within the vegetation structures. Grid positions of the sampling points were then noted. The sample size (in area occupied by natural vegetation), n, was determined by the equation provided by Webster & Oliver (1990). The number of sampling points in each sampled vegetation structure
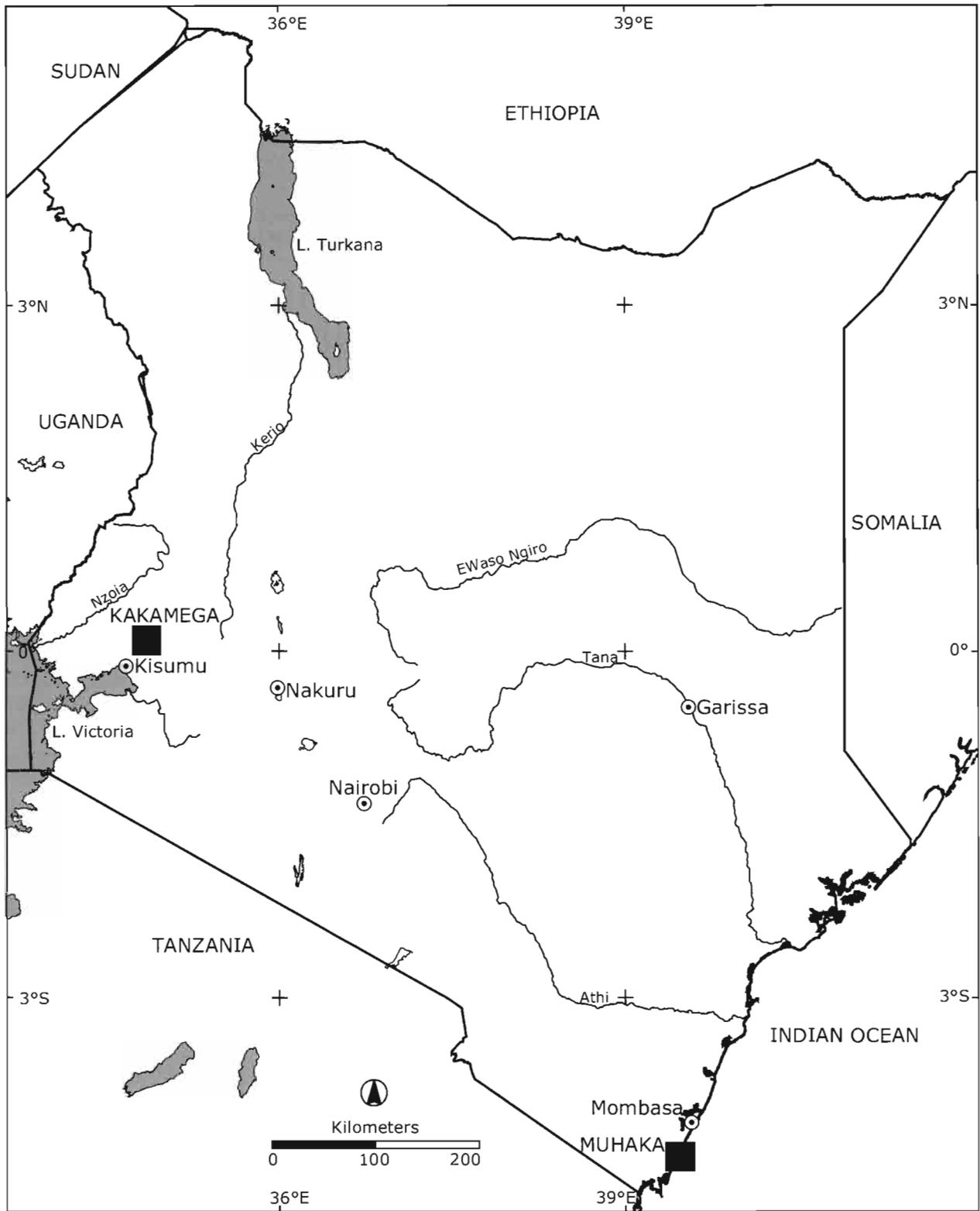
Figure 1
Map of Kenya showing study locations.

was proportional to the relative size of each structure (tab. 1). The sample size equation was as follows;

$$n = (z^2/I^2)pq$$

where n = sample size, I = permitted error (0.1), z = confidence interval (1.64); p = probability of area covered by wild host plants (11.7%) and q = probability of area not covered by wild hosts (maize, tea and natural forest) (88.3) for Kakamega

$$n = (1.64^2/0.1^2) \times 0.117 \times 0.883 = 28$$

For Muhaka; p = 88.7% and q = 11.3% whereby

$$n = (1.64^2/0.1^2) \times 0.887 \times 0.113 = 27$$

Vegetation structures; Forest Glade (FG) and Forest Corridor Vegetation (FCV) in Kakamega had only one sampling point due to their small size. The same was also realised in Roadside Vegetation (RV) in Muhaka. Therefore it became necessary to increase sampling points in such structures to a minimum of three per structure for better statistical analysis. This added to a total of 31 sampling points instead of 28 comprising 93 transect lines in each study location. The noted grid positions of the sampling points were fed into a portable Geographical Positioning System (GPS) kit, Meridian-GPS Magellan, which was used later to identify the points in the field.

Measurement procedure

Transect intercept method of sampling vegetation as described by Grieg-Smith (1983) was employed. At the beginning of
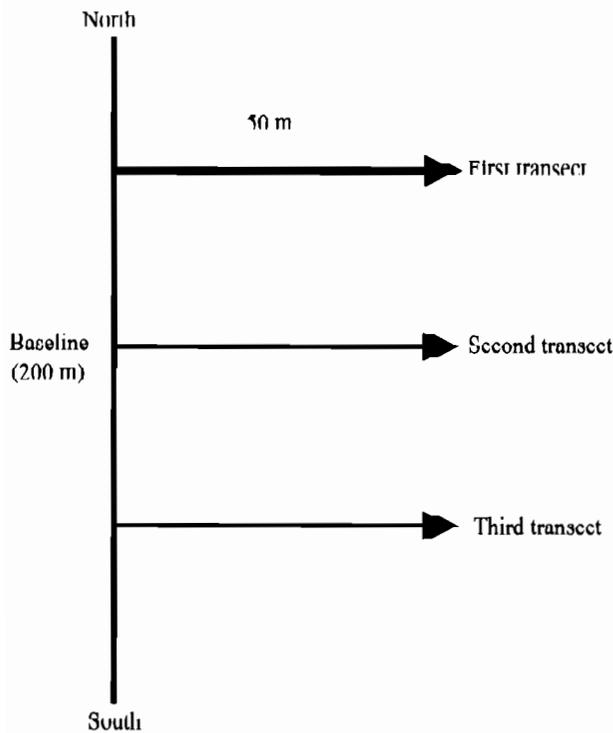

Figure 2
Establishment of baseline and transect lines where sampling took place.

each sampling point, a line, referred to as baseline stretching 200 m orienting in a South-North direction was established. The baseline (200 m) was divided into ten equal parts (marks) starting from 0 to the 10th mark (the end of 200 m length). Three sets of numbers from the ten designated marks were randomly generated and then allocated to each baseline. The set of numbers formed the starting points where line transects were established from the baseline. Three line transects (transects 1, 2, 3) of 50 m each were then established starting at the marked points, running perpendicular to the baseline and parallel to one another in a west-east direction (fig. 2). Sampling was done along the line transects and the exercise was repeated in all the sampling points.

All species belonging to Cyperaceae, Poaceae and Typhaceae intercepting the transects were recorded (Phillips 1995; Frits van Oudtshoorn 2004). Where species identification was not possible in the field, voucher specimen were collected and taken to the herbarium of the University of Nairobi for identification. Crown cover (the proportion of the ground cover occupied by a perpendicular projection of the aerial parts of the individual plant species) of each plant intercepting the transect was recorded (Greig-Smith 1983). The intercept lengths in the whole vegetation structures were summed. This was then divided by the total length of the transect and converted to percentage to give the proportion occupied by each species in that vegetation structure. This gave the percentage cover. Relative abundance was achieved by dividing percentage cover of each plant species by the total percentage cover of all the plant species in a particular vegetation structure. This was used to calculate diversity index. List of wild host plants of stem borer pests was extracted from the list provided by Le Ru et al. (2006a, 2006b).

Data Analysis

Diversity and abundance of wild host plants sampled in Kakamega and Muhaka sites were computed using Shannon-Weaver diversity index (H) (Magurran 1988):

$$H = - \Sigma \, Pi\ln Pi \text{ where } Pi = Ni/N \text{ (relative abundance)}$$

H: Shannon's diversity index, i : host plant species, Pi: proportion of N made up of the ith species, N: total crown cover of all wild host plant species in a particular vegetation structure, Ni: total crown cover of individual wild host plant species, ln : natural logarithm. The resulting product was multiplied by -1 to make negative figures positive.

A t-test was used to compare the diversity indices (Magurran 1988) between vegetation structures within a location and between seasons within the same vegetation structures.

$$t = (H_1 - H_2)/(VarH_1 + VarH_2)^{1/2}$$

where H_I is the diversity in structure 1, Var H_I its variance and N_I total crown cover.

$$Var\,H = \{[\Sigma pi(\ln pi)^2 - (\Sigma pi\ln pi)^2]/N\} - [(S - 1)/2N^2]$$

The degrees of freedom was calculated using the equation,

$$df = [(VarH_1 + VarH_2)^2]/\{[(VarH_1)^2/N_1] + [(VarH_2)^2/N_2]\},$$

where S: plant species richness

Results

Diversity and abundance of wild host of stem borers

Kakamega

There were four distinct vegetation structures in this location: Forest and Riverbank Vegetation (FRV), Between Cultivated (BC), Forest Glade (FG) and Forest Corridor Vegetation (FCV) (tab. 1). A total of 20 wild host plant species of stem borers were recorded with cropping season recording 18 while non-cropping season had 16 (tabs. 2 & 3). However, there was no variation in species diversity between the two seasons ($t_{37} = 0.75; p > 0.05$). The H values were 2 and 1.8 for cropping season and non-cropping season, respectively. Species richness did not vary significantly between vegetation structures (tab. 4). During the cropping season, the number of host species varied between 6 and 13 (6 species recorded in both FG and FCV and 13 in FRV). The species richness was proportional to the area (size) of the vegetation structure though BC, where cultivation was taking place, and FRV, that had wet-micro-climate, seemed to favour growth of host plant. During the non-cropping season, the vegetation structure, BC, had the highest number of hosts (11 species) followed by FRV (10 species) (tabs. 2 & 3). The variation in host plant distribution between the seasons in the same structure was attributed to their absence along some of the transect lines. The highest indices of 1.95 and 1.5 were recorded in FRV during the cropping and non-cropping season respectively. In contrast, the lowest diversity indices of 0.89 and 0.77 were recorded in FG during cropping season and non-cropping season respectively. The other structures showed intermediate values (tab. 4).

About 43% of the surface area surveyed was under cultivation (mainly maize), which was relatively high compared to total wild host species surface cover during the cropping season (2.2%) and non-cropping season (2.6%) (tab. 3). The relative abundance of wild host plants did not vary with the seasons ($t_{37} = 0.75; p > 0.05$) (tab. 4). By contrast, host plants surface cover varied between 0.11 and 0.93% among the vegetation structures during the cropping season and 0.07 and 1.1% during the non-cropping season. The highest cover was observed in the Forest edge and Riverbank Vegetation (FRV) where wild host plants had surface cover 0.7% during the cropping and 1.1% during the non-cropping season. This was a complete opposite with other structures where surface cover was higher during the cropping and lower during the non-cropping season (tab. 3).

Muhaka

There were five vegetation structures: Mixed Vegetation (MV), Open Grassland (OG), Palm Vegetation (PV), Natural Forest and Edge (NF&E) and Roadside Vegetation (RV) in this location (tab. 1). A total of 16 wild hosts species were recorded with a marked difference in species richness between the seasons (16 species during the cropping season and 7 during the non-cropping season) (tabs. 3 & 5). A significant variation in species diversity was recorded between the seasons ($t_{46} = 2.89; p < 0.05$), with higher diversity index during the cropping season than the non-cropping season (tab. 4). Wild host species richness varied between 6 and 11 during the cropping season with MV recording the highest (11) among the vegetation structures. The number reduced during the non-cropping season with MV still recording the highest (7) (tab. 3). Palm Vegetation (PV) and RV showed significant variation in species diversity compared to the other structures during the cropping season [MV-PV ($t_{45} = 3.39; p < 0.05$), OG-PV ($t_{45} = 2.75; p < 0.05$), RV-OG ($t_{43} = 2.17; p < 0.05$), RV-PV ($t_{36} = 5.28; p < 0.05$), RV-NF&E ($t_{55} = 3.64; p < 0.05$)]. During non-cropping season, a significant difference was observed between MV and

Table 1. Area covered (km²) and percentage surface cover by each vegetation structure in Kakamega and Muhaka. In parenthesis is the number of sampling points allocated to the vegetation structures inhabited by wild host plants.

Kakamega	Land cover vegetation structure	Area (km²)	Cover %
	Maize (M)	9.16	43.13
	Tea (T)	0.42	1.96
	Natural Forest (NF)	9.17	43.18
	Between Cultivated (BC)	0.60	2.83 (8)
	Forest edge and Riverbank vegetation (FRV)	1.38	6.48 (15)
	Forest Glade (FG)	0.34	1.62 (4)
	Forest Corridor Vegetation (FCV)	0.17	0.81 (4)
	Total Area	**21.25**	**100 (31)**
Muhaka	Mixed Vegetation (MV)	9.69	44.37 (13)
	Palm Vegetation (PV)	3.13	16.22 (5)
	Open Quary (OQ)	0.05	0.24
	Buildings (B)	0.07	0.36
	Roadside Vegetation (RV)	0.10	0.54 (3)
	Natural Forest and Edge (NF&E)	1.56	8.09 (4)
	Open Grassland (OG)	3.76	19.69 (6)
	Cultivated Vegetation (CV)	2.06	10.69
	Total Area	**19.30**	**100 (31)**

Table 2. Total relative cover (%) and relative cover of each wild host plant species of lepidopteran stem borers in the four different vegetation structures in Kakamega during the cropping and non-cropping seasons.
The wild host plants of *B. fusca* are in bold.

	Plant species	FRV	BC	FG	FCV	Cover %
Cropping season	*Cyperus dives* Delile	0.09	0.15	0	0	0.08
	Cyperus distans L.	0.50	0.25	0	0	0.31
	Cyperus dichrostachyus A. Richard	0	0.13	0	0	0.03
	Scleria racemosa Poiret	0.30	0.90	0	0	0.38
	Brachiaria brizantha (A. Richard) Stapf	1.08	1.47	42.52	1.67	6.60
	Cynodon dactylon (L.) Persoon	4.94	5.65	3.53	0.80	4.41
	Digitaria milanjiana (Rendle) Stapf	0	0	0.07	0	0.01
	Hyparrhenia diplandra (Hackel) Stapf	0.67	0	0	0	0.32
	Hyparrhenia rufa (Nees) Stapf	0.46	1.25	0	0	0.54
	Panicum maximum Jacquin	0.23	0.15	4.62	2.10	1.02
	Pennisetum macrourum Trinius	0.64	0	0	0	0.31
	Pennisetum purpureum Schumacher	0.58	2.33	0	0	0.88
	Pennisetum clandestinum (Chiovenda) Hochstetter	0	0	0.50	0.20	0.09
	Pennisetum trachyphyllum Pilger	0	0	0	2.67	0.34
	Setaria megaphylla (Steudel) T. Durand & Schinz	0.88	3.08	0	6.30	2.04
	Setaria sphacelata (Schumacher) Moss	0.18	0	0	0	0.09
	Sorghum arundinaceum (Desvaux) Stapf	0.30	0	6.43	0	0.98
	Typha domingensis Persoon	0	0.67	0	0	0.17
	Average cover in respective structures	10.84	16.03	57.67	13.73	18.60
Non-cropping season	*Cyperus dives* Delile	0	0.09	0	0	0.03
	Cyperus distans L.	0	0.16	0	0	0.06
	Cyperus dichrostachyus A. Richard	0	0.27	0	0	0.10
	Scleria racemosa Poiret	0.07	0.09	0.09	0	0.07
	Brachiaria brizantha (A. Richard) Stapf	0.70	0.17	26.93	0.74	5.427
	Cynodon dactylon (L.) Persoon	7.56	3.25	1.78	0.14	6.57
	Cymbopogon nardus (L.) Rendle	0.41	0.300	3.47	0	1.01
	Digitaria milanjiana (Rendle) Stapf	4.25	6.75	0	1.00	5.36
	Echinochloa pyramidalis (Lamark) Hitchcock & Chase	0.07	0	0	0	0.05
	Panicum maximum Jacquin	0	0	0.79	0.67	0.20
	Pennisetum macrourum Trinius	0.45	0	0	0.17	0.32
	Pennisetum purpureum Schumacher	0.27	0.54	0	0	0.37
	Setaria megaphylla (Steudel) T. Durand & Schinz	2.14	1.33	0	1.44	2.04
	Pennisetum trachyphyllum Pilger	0	0	0	4.05	0.36
	Sorghum arundinaceum (Desvaux) Stapf	0.37	0	0.85	0.73	0.47
	Typha domingensis Persoon	0	0.37	0	0	0.13
	Average cover in respective structures	**16.51**	**13.30**	**33.90**	**8.95**	**22.60**

FRV: Forest and Riverbank Vegetation; BC: Between Cultivation; FG: Forest Glade; FCV: Forest Corridor Vegetation.

NF&E (t_{32} = 2.07; $p < 0.05$) and between MV and RV (t_{30} = 2.38; $p < 0.05$) (tab. 4).

The relative abundance of wild hosts varied significantly between the two seasons (t_{46} = 2.89; $p < 0.05$) (tab. 4). The total wild host species surface cover was two times (23%) higher than maize (10.7%) during the cropping season than during the non-cropping season. Maize plots were mainly found in OG and MV structures where wild host species constituted about 4.9 and 12.1%, respectively, during

the cropping season, and 2.7 and 8.2%, respectively, during the non-cropping season (tab. 3).

Wild hosts of *Busseola fusca* and *Chilo partellus*

Three wild hosts plant species [*Sorghum arundinaceum* (Desvaux) Stapf, *Setaria megaphylla* (Steudel) T. Durand & Schinz and *Pennisetum purpureum* Schumacher] of *B. fusca* were recorded in both seasons in Kakamega. In Muhaka 3 wild hosts [*Panicum. maximum* Jacquin, *S. arundinaceum* and

Table 3. Surface cover (%) and species richness of host plants in the different vegetation structures during cropping season (CS) and non-cropping season (NCS) in Kakamega and Muhaka.
In parenthesis is the surface cover (%) and species richness of B. fusca and C. partellus host plants

Vegetation structures	Seasonal variation			
	Surface cover (%)		Species richness	
Kakamega	CS	NCS	CS	NCS
Cultivated (maize) habitat	43.3	0		
Uncultivated (wild host) habitat	2.2(0.46)	2.6(0.34)	18(3)	16(3)
FRV	0.7(0.11)	1.1(0.18)	13(3)	10(3)
BC	0.46(0.15)	0.37(0.05)	11(2)	11(2)
FG	0.93(0.10)	0.55(0.01)	6(1)	6(1)
FCV	0.11(0.05)	0.07(0.02)	6(1)	8(2)
Muhaka	CS	NCS	CS	NCS
Cultivated (maize) habitat	10.69	0		
Uncultivated (wild host) habitat	23(2.17)	17.9(1)	15(3)	7(1)
MV	12.09(1.3)	8.24(0.46)	11(1)	7(0)
OG	4.9(0.45)	2.7(0.18)	10(0)	4(0)
RV	0.13(0.03)	0.059(0.01)	11(2)	2(0)
PV	3.44(0.02)	5.17(0.22)	6(0)	4(0)
NF&E	2.53(0.16)	2.13(0.02)	8(1)	3(0)

FRV: Forest and Riverbank Vegetation; BC: Between Cultivation; FG: Forest Glade; FCV: Forest Corridor Vegetation; MV: Mixed Vegetation; OG: Open Grassland; PV: Palm Vegetation; NF&E: Natural Forest & Edge; RV: Roadside Vegetation.

Table 4. t-statistics for difference between vegetation structures for within and between seasons in Kakamega and Muhaka study locations.

Kakamega	Cropping Season (CS)							Non-cropping Season (NCS)							Between seasons	
	BC		FG		FCV			BC		FG		FCV				
	df	t	df	t	df	t	H_{CS}	df	t	df	t	df	t	H_{NCS}	df	t
FRV	19	0.27ns	17	3.63*	18	1.66ns	1.95	28	0.41ns	37	2.83*	24	0.30ns	1.50	23	1.33ns
BC			39	4.65*	30	1.86ns	1.87			27	2.17*	23	0.71ns	1.49	23	1.23ns
FG					36	2.70*	0.89					24	3.32*	0.77	52	0.44ns
FCV							1.45							1.64	18	0.77ns
Total cover							2.00							1.81	37	0.75ns

Muhaka	Cropping Season (CS)									Non-cropping Season (NCS)									Between seasons	
	OG		PV		NF&E		RV			OG		PV		NF&E		RV				
	df	t	df	t	df	t	df	t	H_{CS}	df	t	df	t	df	t	df	t	H_{NCS}	df	t
MV	52	0.51ns	45	3.39*	55	1.51ns	48	1.67ns	1.66	30	1.05ns	35	1.03ns	32	2.07*	30	2.38*	1.08	43	2.34*
OG			45	2.75*	50	0.89ns	43	2.17*	1.53			25	0.25ns	22	0.68ns	24	1.08ns	0.80	34	2.72*
PV					42	1.19ns	36	5.28*	0.80					58	1.31ns	28	1.73ns	0.86	23	0.29ns
NF&E							55	3.64*	1.33							24	0.61ns	0.65	56	3.82*
RV									2.00									0.54	27	7.89*
Total cover									1.67									0.95	46	2.89*

H: Shannon diversity index; df: degrees of freedom; t: t-test values; ns: Diversity not significantly different; *: Diversity significantly different at P < 0.05.
FRV: Forest and Riverbank Vegetation, BC: Between Cultivation, FG: Forest Glade, FCV: Forest Corridor Vegetation.
MV: Mixed Vegetation, OG: Open Grassland, PV: Palm Vegetation, NF&E: Natural Forest & Edge, RV: Roadside Vegetation.

Rottboellia cochinchinensis (Loureiro) Clayton] of *C. partellus* were recorded during the cropping season and only *P. maximum* during the non-cropping season. Hosts of *B. fusca* varied between 1 and 3 among vegetation structures in both cropping and non-cropping seasons, and 1 in FG and 3 in FRV during cropping and non-cropping seasons in Kakamega. In Muhaka, wild host of *C. partellus* varied between 1 and 3 among vegetation structures during the cropping season with RV having the highest (3). During the non-cropping season, only *P. maximum* was present in all structures (tab. 5).

In Kakamega, surface cover of wild host plant species of *B. fusca* was about 0.46 and 0.34% during the cropping and non-cropping seasons respectively while in Muhaka wild hosts of *C. partellus* constituted 2.17 and 1% during the two seasons. However, surface cover of *B. fusca* hosts varied between 0.05 and 0.15% during the cropping and between 0.01 and 0.18% during the non-cropping season among vegetation structures. Hosts of *C. partellus* in OG and MV

vegetation structures varied between 0.45 and 1.3% respectively during the cropping season and 0.18 and 0.46% respectively during the non-cropping season (tab. 3).

Discussion

In Kakamega no difference in wild host plant species richness and relative abundance was found between both cropping and non-cropping seasons suggesting continuous presence of host plants throughout the year. This further suggests that the natural habitat could support the population of *Busseola fusca* during the intercropping period in this location. However, *B. fusca* has been reported to enter diapause at the end of cropping season (Kfir 1991). In addition, among the wild plant species identified in this study location only three plants (*Sorghum arundinaceum*, *Setaria megaphylla* and *Pennisetum purpureum*) were identified as hosts of *B. fusca* (Le Rü *et al.* 2006a, 2006b). Coupled with the limited host range of this pest, dense human population is putting more pressure on the remaining

Table 5. Total relative cover (cover %) and relative cover of each wild host plant species of lepidopteran stem borers in the four different vegetation structures in Muhaka during the cropping and non-cropping seasons. The wild host plants of *C. partellus* are in bold.

	Plant species	MV	OG	PV	NF&E	RV	cover %
Cropping season	*Cyperus exaltatus* Retz	1.61	1.91	1.87	6.83	0.98	2.32
	Cyperus dives Delile	0.10	0	0	0	0	0.04
	Cyperus prolifer Lamark	0	0.02	0	0	0	0.01
	Brachiaria brizantha (A. Richard) Stapf	1.94	0.22	0.11	0	0.40	0.912
	Cenchrus ciliaris L.	0.05	0	0	0	2.62	0.28
	Cynodon dactylon (L.) Persoon	0	0	0	0	6.67	0.645
	Digitaria milanjiana (Rendle) Stapf	8.94	12.64	15.77	15.47	3.11	11.04
	Echinochloa haploclada (Stapf) Stapf	0	0.56	0	0	0	0.11
	Eriochloa meyeriana (Nees) Pilger Engler & Prantl	0.46	1.69	0	0	0	0.52
	Hyperthelia dissoluta (Steudel) W.D. Clayton	9.95	4.78	3.24	6.03	2.67	6.66
	***Panicum maximum* Jacquin**	**2.40**	**2.33**	**0.11**	**1.83**	**4.22**	**2.12**
	Panicum merkeri Mez	0.61	0.09	0	0.50	0.22	0.36
	Pennisetum polystachyon (L.) Schultes	0.66	0.89	0.11	0.33	1.56	0.66
	***Rottboellia cochinchinensis* (Loureiro) Clayton**	**0.53**	**0**	**0**	**0.13**	**0.44**	**0.28**
	Setaria sphacelata (Schumacher) Moss	0	0	0	0.13	0	0.02
	***Sorghum arundinaceum* (Desvaux) Stapf**	**0**	**0**	**0**	**0**	**0.40**	**0.039**
	Average cover in respective structures	27.25	25.13	21.20	31.27	23.29	26.00
Non-cropping season	*Cyperus exaltatus* Retzius	0.65	0.12	0.48	0	0	0.40
	Cyperus prolifer Lamark	0.05	0	0	0	0	0.02
	Digitaria milanjiana (Rendle) Stapf	10.02	9.76	19.93	18.78	0	12.23
	Hyperthelia dissoluta (Steudel) W.D. Clayton	6.43	2.94	10.08	7.24	8.47	6.37
	Panicum merkeri Mez	0.12	0	0	0	0	0.05
	***Panicum maximum* Jacquin**	**1.14**	**0.947**	**1.39**	**0.29**	**2.50**	**1.09**
	Pennisetum polystachion (L.) Schultes	0.17	0	0	0	0	0.08
	Average cover in respective structures	18.57	13.77	31.88	26.31	10.97	20.24

MV: Mixed Vegetation. OG: Open Grassland. PV: Palm Vegetation. NF&E: Natural Forest & Edge. RV: Roadside Vegetation.

land resulting in constant destruction of wild habitat (Otieno *pers obs*). According to Altieri (1991) and Tscharntke & Brandl (2003) destruction of wild habitat may disrupt the plant-herbivore interaction and thus affect the pest population in the wild habitat.

In Muhaka location, there was variation in wild host species richness and relative abundance between the seasons though the results show higher surface cover of wild host plants relative to maize. However, among the hosts recorded, only three (*Panicum maximum, S. arundinaceum* and *Rottboellia cochinchinensis*) were identified as host plants of *Chilo partellus* (Le Rü *et al* this issue). *P. maximum* was the main alternate host of *C. partellus* present in all vegetation structures in both seasons. The other hosts (*S. arundinaceum* and *R. cochinchinensis*) were mainly found at the edge of cultivated fields confirming that farming practice can favour the maintenance of alternative host plants for stem borer pests during the non-cropping season (Rebe & Van den Berg 2001). However, uncontrolled burning of wild habitats during the dry season to clear land in preparation for the cropping season, probably was responsible for the low abundance of wild hosts of the pest in Muhaka location in addition to the long dry season.

A similar study carried out in Kitale in 2003 on the abundance and diversity of alternative hosts plants of stem borers reported 14 wild hosts of cereal pests (*B. fusca* and *C. partellus*) (Kanya *et al.*, 2005). This was based on earlier report by Polaszek & Khan (1998) that *B. fusca* and *C. partellus* are polyphagous. The high number of host plants reported earlier was most probably due to misidentification of the stem borers recovered from the wild habitat (Le Rü *et al.* 2006a, 2006b). However, Kanya *et al* (2005) reported wild host abundance of < 10% compared to maize 95%. Interaction between vegetation structure and movement patterns of insects ultimately affects population dynamics in a heterogeneous landscape (Burel *et al.* 2000). Thus, the absence or presence of alternate hosts alone might not account for pest outbreaks in the cultivated fields, and hence, there is need to study the movement patterns of the stem borers as well.

The limited information available on movement of adult moths suggests that most of the population moves relatively short distances (less than 100 m) within the crop or the adjacent vegetation though reports indicate that *B. fusca* is likely to fly long distance under optimum conditions (Fitt *et al.* 2004). However, it is worth noting that *B. fusca* recovered in Kakamega from *S. megaphylla* did not develop to up to adult moths in the artificial diet under laboratory conditions (contrary

to *B. fusca* recovered from *S. arundinaceum*) (Le Rü, *pers. obs.*). This could be an indication that the *B. fusca* population belonging to this location might be divided into different compartments with very low exchange between cultivated and non-cultivated habitats. Future prospects need to investigate the structure of *B. fusca* populations in Kakamega and to quantify the exchange between the different habitats.

On these understandings, the importance of wild habitats as reservoir of stem borer pests cannot be estimated until the information on the dispersal potential of stem borers is generated. However, our study and that done by Kanya *et al.* (2005) showed that the area covered by wild host plants was below 10% and therefore inadequate to sustain susceptible stem borers as it is recommended that 20-50% to be non-transgenic plants (Fitt *et al.* 2004).

Contrary to the previous reports (Polaszek & Khan, 1998), this study shows that the host range of *B. fusca* and *C. partellus* is limited both in number and abundance. Nonetheless, it is not clear whether the observed low abundance may facilitate the carry-over of these stem borer pest species between the growing seasons. Probably, these species invading cereal crops come either from other areas or from the maize/sorghum residues present in the crop fields. Attempt should thus be made to study whether traditional farming practices contributes to carry-over of the pests.

References

Altieri M. A. 1991. Increasing biodiversity to improve insect pest management in agro-ecosystems, p. 165-182 *in*: **Hawksworth. D. L** (eds), *Biodiversity of microorganisms and invertebrates: Its Role in sustainable agriculture*, Wallingford, UK: CAB International.

Arc View 3.2. 1992. Environmental Systems Research Institute, New York.

Bowden J. 1954. The Stem-borer problem in Tropical crops, p. 104-107 *in: Report 6th Commonwealth Entomological Conference*, Commonwealth Institute of Entomology, London

Burel F., Baudry J., Delettre Y., Petit S., Morvan N. 2000. Relating insects movement to farming systems in dynamic landscapes, p. 5-32 *in*: **Ekbom, B., Irwin, M. E. and Robert, Y.** (eds), *Interchanges of insects between agricultural and surrounding landscapes.* Kluwer Academic Publishers, Netherlands.

Fitt G. P., Andow D. A., Carrière Y., Moar W.J., Schuler T. H., Omoto C., Kanya J., Okech M. A., Arama P., Maniania N. K. 2004. Resistance risks and management associated with *Bt* Maize in Kenya, p. 209-250 *in*: **Hilbeck A., Andow D. A.** (eds.), *Environmental risk assessment of genetically modified organisms.* CABI, London, UK.

Frits van Oudtshoorn. 2004. *Guide to grasses of Southern Africa*. Briza, Pretoria, SA, 288 p.

Guofa Z., Overholt W. A., Mochiah M. B. 2002. Changes in the distribution of lepidopteran maize stemborers in Kenya from 1950's to 1990's. *Insect Science and its Application* 21: 395-402.

Grieg-Smith P. 1983. *Quantitative plant ecology*. 3rd edition, Blackwell Scientific Publication, Oxford, England, 359 p.

Guihéneuf Y. 2004. *Characterisation of wild and cultivated habitat in two ecological areas in Kenya*. Mémoire de D.E.S.S., Université de Paris XII, Paris, 52 p.

Haile A., Hofsvang T. 2001. Survey of lepidopterous stemborer pests of sorghum, maize and pearl millet in Eritrea. *Crop protection* 20: 151-157.

Ingram W. R. 1958. The lepidopterous stalk borers associated with graminae in Uganda. *Bulletin of Entomological research* 49: 367-383.

Kanya J. I., Ngi-song A. J., Setamou M. F., Overholt W., Ochora J. and Osir E. O. 2005. Diversity of alternative hosts of maize stemborers in Trans-Nzoia district of Kenya. *Environmental Biosafety Research* 3: 159-168.

Khan Z. R., Lwande W., Ampong-Nyarko K. 1997. *Use of wild habitat in the management of cereal stemborers*. 1995-1997 ICIPE Annual Scientific Report, Nairobi, Kenya.

Kfir R. 1991. Effects of diapause on development and reproduction of the stemborers *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Pyralidae). *Journal of Economic Entomology*. 84: 1677-1680.

Kokwaro J. O. 1988. Conservation Status of the Kakamega Forest in Kenya: The eastern most relic of the equatorial rain forest. Monograph in systematic botany. *Missouri Botanical Garden* 25: 471-489.

Le Rü B.P., Ong'amo G.O., Moyal P., Ngala L., Musyoka B., Abdullah Z., Cugala D., Defabachew B., Haile T.A., Kauma Matama T., Lada V.Y., Negassi B., Pallangyo K., Ravololonandrianina J., Sidumo A., Omwega C., Schulthess F., Calatayud P.-A., Silvain J.F. 2006a. Diversity of lepidopteran stem borers on monocotyledonous plants in eastern Africa and the islands of Madagascar and Zanzibar revisited. *Bulletin of Entomological Research* 96: 1-9.

Le Rü B.P., Ong'amo G.O., Moyal P., Muchugu E., Ngala L., Musyoka B., Abdullah Z., Matama-Kauma T., Lada V.Y., Pallangyo B.,

Omwega C.O., Schulthess F., Calatayud P.-A., Silvain J.-F. 2006b. Geographic distribution and host plant ranges of East African noctuid stem borers. *Annales de la Société Entomologique de France (N.S.)* 42(3-4): 353-361.

Magurran A. E. 1988. *Ecological diversity and its measurement*. Princeton University press, Princeton, 179 p.

Nye I. W. B. 1960. The insect pests of graminaceous crops in East Africa. *Colonial Research Studies* 31: 1-48.

Ong'amo G. O. 2005. *An assessment of lepidopteran stemborer species distribution in relation to diversity of wild host grasses in maize and sorghum growing zones in Kenya*. MSc thesis, Kenyatta University. Nairobi, Kenya, 79 p.

Phillips S. 1995. *Flora of Ethiopia and Eritrea Volume 7. Poaceae (Gramineae)*. Hedberg I., Edwards S. (eds). National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia, 398 p.

Polaszek A., Khan Z. R. 1998. Host plants, p. 5-23 *in*: Polaszek. A (ed), *African Cereal stemborers: economic importance, taxonomy, natural enemies and control*. CAB International 1998. University Press, Cambridge, UK.

Randriamananoro J. J. 1996. *Behaviour and Physiological responses of the cereal stalk borer Busseola fusca Fuller (Lepidoptera: Noctuidae) to selected cultivated and wild host plants*. Ph.D. Dissertation, River State University of Science and Technology, Port Harcourt, Nigeria, 141 p.

Rebe M., Van den Berg J. 2001. *Wild host plants of lepidopterous stem borers in Limpopo and Northern Mpumalanga Provinces of South Africa*. Pietermaritzburg, 63 p.

Seshu Reddy K. V. 1983. Sorghum stem borers in East Africa. *Insect Science and its Application* 4: 33-39.

Seshu Reddy K. V. 1989. Sorghum stem borers in Eastern Africa, p 33-40. *Proceedings of the International Workshop on sorghum stem borers, 17th-20th November, 1987*, ICRISAT, Patancheru, India.

Tscharntke T., Brandl R. 2003. Plant-insect interactions in fragmented landscapes. *Annual Review of Entomology* 49: 405-430.

Webster R., Oliver M. A. 1990. *Statistical methods in soil and land resource survey*. Oxford University Press. New York, 316 p.

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The temporal correlation and spatial synchrony in the stemborer and parasitoid system of Coast Kenya with climate effects

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Abstract. The spatial synchrony and the temporal auto-correlationship of the exotic stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and the indigenous *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Chilo orichalcociliellus* (Strand) (Lepidoptera: Crambidae), and cross-correlationship with their indigenous and introduced larval parasitoids *Cotesia sesamiae* (Cameron) and *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was studied using 3-year data collected in coastal Kenya. An autoregressive model was used to study the effect of climatic stochasticity or population density-dependent factors on stemborer populations. It appeared that rainfall did have a direct impact on stemborers in the south coast and an indirect one in the north coast. Spatial non-parametric correlation functions (SNCF) and cross-correlation functions (SNCCF) were applied for spatial synchrony analysis. The regional synchrony of *Ch. partellus* and *S. calamistis* decreased and that of *Ch. orichalcociliellus* increased after the introduction of *Co. flavipes*. The positive cross-correlation coefficient between stemborers and parasitoids suggests a synchrony between the pest and its natural enemy.

Résumé. Corrélation temporelle et synchronie spatiale avec les effets climatiques dans le système foreur et parasitoïde dans la région côtière du Kenya. Le degré de synchronie spatiale et la relation d'auto corrélation temporelle de la distribution d'une espèce de foreur de graminées exotique *Chilo partellus* (Swinhoe) (Lepidoptera : Crambidae) avec celles indigènes *Sesamia calamistis* Hampson (Lepidoptera : Noctuidae) et *Chilo orichalcociliellus* (Strand) (Lepidoptera : Crambidae), ainsi que la relation croisée d'auto correlation avec les populations d'espèce de parasitoïde larvaire indigène et introduite, *Cotesia sesamiae* (Cameron) et *Cotesia flavipes* Cameron (Hymenoptera : Braconidae), ont été étudiés à partir de données collectées sur trois ans dans la région côtière du Kenya. Un modèle d'auto régression a été utilisé afin d'étudier l'effet stochastique du climat ou les facteurs dépendants de la densité de population sur les foreurs. Il est apparu que la pluie avait un impact direct au sud ou indirect au nord de la région étudiée sur les populations de foreurs. Des fonctions non-paramétriques de corrélation spatiale et croisée ont été appliquées pour l'analyse du degré de synchronie spatiale. Le degré de synchronie régionale des populations de *Ch. partellus* et de celles de *S. calamistis* a diminué alors qu'il a augmenté pour celles de *Ch. orichalcociliellus* après l'introduction de *Co. flavipes*. Le coefficient positif d'auto corrélation entre les populations de foreurs et celles des parasitoïdes suggère un certain degré de synchronie entre les insectes ravageurs et leurs ennemis naturels.

Keywords: Stemborer, parasitoid, correlation, spatial synchrony, climate.

There are two types of factors influencing the population dynamics of insects: endogenous factors, which generate the first- and second-order feedback structure, and exogenous factors, e.g. climate. In populations where fluctuations are affected by trophic interactions such as predator-prey or host-parasitoid systems, the correlated climatic effects either operate via density-dependent mechanisms, whereby strong density dependence would enhance the synchrony, or more directly on population abundance, moving the

populations towards a synchronous phase (Bjørnstad & Bascompte 2001; Lima *et al.* 2002; Stenseth *et al.* 2002; Cattadori *et al.* 2005), which means that correlated population fluctuates over localized or wide-scaled geographical areas (Ranta *et al.* 1995; Hudson & Cattadori 1999). Furthermore, dispersal or the mobility of the host and parasitoid determine the synchrony of the population (Bjørnstad *et al.* 1999).

Chilo partellus (Swinhoe) (Lepidoptera: Crambidae), an exotic stemborer species invaded Africa sometimes before the 1930s (Tams 1932). It has since then become the most damaging stemborer species of maize and sorghum in the lowlands of East and Southern Africa (ESA) (Overholt *et al.* 1997; Zhou *et al.* 2001a). Parasitism of the most common indigenous larval

parasitoids, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), typically never exceeds 5% (Sallam *et al.* 1999). Therefore, the exotic *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was introduced from Asia in 1991 and it was released in the coastal Kenya in 1993 (Overholt *et al.* 1994a). By 2000, it has reduced *Ch. partellus* density by 57% and increased maize yields by 10-15% (Zhou *et al.* 2001a). Since then, the parasitoid was released and became permanently established in nine countries in ESA (Omwega C., ICIPE, *pers. com.*). There are several indigenous stemborer species in the region, whose importance vary with agro-ecozone, such as *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae) and some are suitable to *Co. flavipes*. Zhou *et al.* (2001a) studied the impact of *Co. flavipes* on the stemborer dynamics in Kenya but without consideration for the impact of climate on stemborer populations or the effect of the introduced natural enemy on the indigenous stemborer species, which would affect the spatial synchrony of stemborers with parasitoids.

In this study, we investigated the exogenous and endogenous factors influencing the population dynamics of *Ch. partellus* and two other major indigenous species in coastal Kenya. The order of the autoregressive model was analyzed using statistical methods by Bjørnstad *et al.* (1999), Bjørnstad & Bolker (2000) and Bjørnstad & Bascompte

Figure 1
The sampling sites in north and south coastal Kenya (circles: stands for all the sampling sites in south coast, and triangles: stands for all the sampling sites in north coast).

(2001) in order to test their spatial synchrony with the indigenous and introduced parasitoids *Co. sesamiae* and *Co. flavipes*, respectively. This method is a new tool for studying theoretical spatial patterns and it has been applied to study forest insect dispersal and outbreak (Bjørnstad *et al.* 2002; Peltonen *et al.* 2002) and predator-prey systems (Tobin & Bjørnstad 2003).

Materials and Methods

Field sites

Field surveys were conducted in coastal Kenya (fig. 1), which was split into the north and the south coast (Zhou *et al.* 2001a). In both regions, *Ch. partellus* is the predominated stemborer species, while the indigenous *Ch. orichalcocilleus* and *S. calamistis* are of minor importance. *Co. flavipes* was released in three sites in 1993 (Zhou & Overholt 2001). There are usually two cropping seasons per annum, the long rainy season (LR) lasting from April-September, and the short rainy season (SR) lasting from October-March. Data were collected monthly from 95 to 1998, except for the SR of 1996. In each field, 20 plants were randomly sampled and dissected (Overholt *et al.* 1994b; Zhou & Overholt 2001; Zhou *et al.* 2001a). Borers collected were reared in the laboratory until adult moth or parasitoid emergence. The total number of borers parasitized by *Co. flavipes* or *Co. sesamiae* per site was recorded. Parasitism was calculated as the percentage of parasitized larvae of the total third and fourth larval instars. Totally 21 sites with 43 time series were used in this study. Time series were split into two phases to compare the effect of parasitism on the spatial synchrony of stemborer populations, i.e., before and after the end of 1996, when parasitism by *Co. flavipes* increased exponentially (Zhou *et al.* 2001a). In addition, for further follow-up the plots were revisited in the short rainy season of 2004 and long rainy season of 2005.

Climatic variability was estimated using the annual precipitation pattern as index calculated as daily rainfall (mm) at Mtwapa and Kwale in the north and south coast, respectively. Temperature was not included because minimum and maximum values did not vary between the two regions (Jiang, *in lit.*).

Statistical analysis

For each stemborer species, the temporal density-dependent effect was assessed by detecting the order of the time series of their monthly growth rates which is the ratio of its density at current month to its density at previous month, using the analysis of time series data of proc ARIMA of SAS (SAS 2000). It is assumed that the stemborer growth rate is a linear combination of its own value at the previous time and as cross-correlation of current or past time values of rainfall. Stepwise regression model with the impact of rainfall was estimated via the Akaike's information criterion (AIC) (Burnham & Anderson 1998). The monthly growth rate of each stemborer species population was calculated as the number at time t divided by the number at t-1. Berryman (2001) stated that the order or number of time lags of the autoregressive model related to the regulatory structure of the tri-trophic system and it reflected the number of functionally different interacting groups.
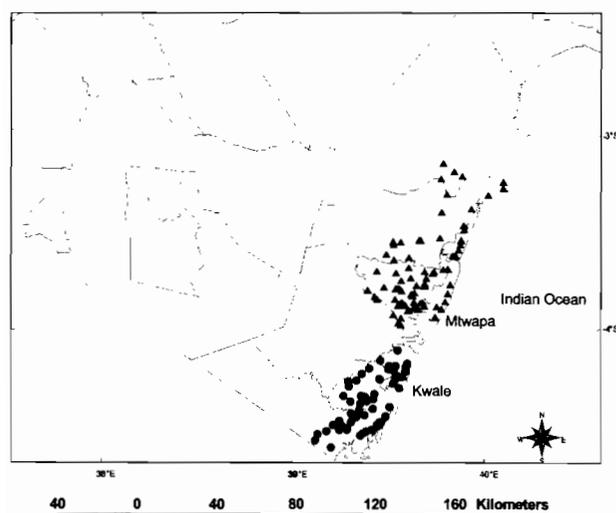
The spatial synchrony of the exotic stemborer, *Ch. partellus*, and the indigenous *S. calamistis* and *Ch. orichalcociliellus*, as well as their spatial cross-correlation with the indigenous and exotic parasitoids *Co. sesamiae* and *Co. flavipes*, respectively, was studied using a non-parametric spatial correlation function (SNCF) and spatial cross-correlation function (SNCCF) (http://asi23.ent.psu.edu/onb1/software.html) (Bjørnstad *et al.* 1999; Bjørnstad & Falck 2001). The program environment was R 2.2.0 (www.r-project.org).

The value of the spatial synchrony represents different spatial pattern of the population. The covariance of the locally stable population is high between the neighboring populations as a result of dispersal, and it decays to the level of the regional synchrony with distance (Bjørnstad *et al.* 1999). The average synchrony is the value of the correlation with the environmental stochasicity; a cyclic population would exhibit a high synchrony throughout the region, while a chaotic population exhibits little synchrony, and the regional noise would not lead to synchronization of the dynamics.

Results

The seasonal population dynamics and parasitism

Chilo partellus was the dominant stemborer species in both the north and south coast. By 2005, the average seasonal population density of *Ch. partellus* in the north coast was reduced to under 0.5/plant (fig. 2a). Densities of *Ch. orichalcociliellus* and *S. calamistis* also declined compared to 1998. Parasitism of *Ch. partellus* by *Co. flavipes* had increased to about 30%, while parasitism of *Co. sesamiae* fluctuated with seasons and it was higher during the short than long rainy season. In the south coast, *Ch. partellus* declined to 0.6/plant by 2005 (fig. 2b). Densities of the other two indigenous stemborer species did not vary much throughout the years. Parasitism of *Co. flavipes* increased to about 20% during the short rainy season of 2004 and then declined again in 2005. Parasitism by *Co. sesamiae* was generally very low.

Density-dependent effects of stemborer populations and the effect of rainfall

Figures 3a & b show the growth rates of each species together with rainfall in the north and south coast. The rainfall pattern in the north and south coast were similar, but the level was higher in the north than south. In the north coast, the monthly growth rates of *Ch. partellus* fit the first and 2nd-order autoregressive (AR) model, and it was significantly correlated with rainfall at *t-2*, i.e., rainfall at 2 months previously. The growth rate of *Ch. orichalcociliellus* also represented a first- and second-order autoregressive model, but it had no relationship with rainfall. Alternatively, its growth rate could also be described by a 1st -order AR
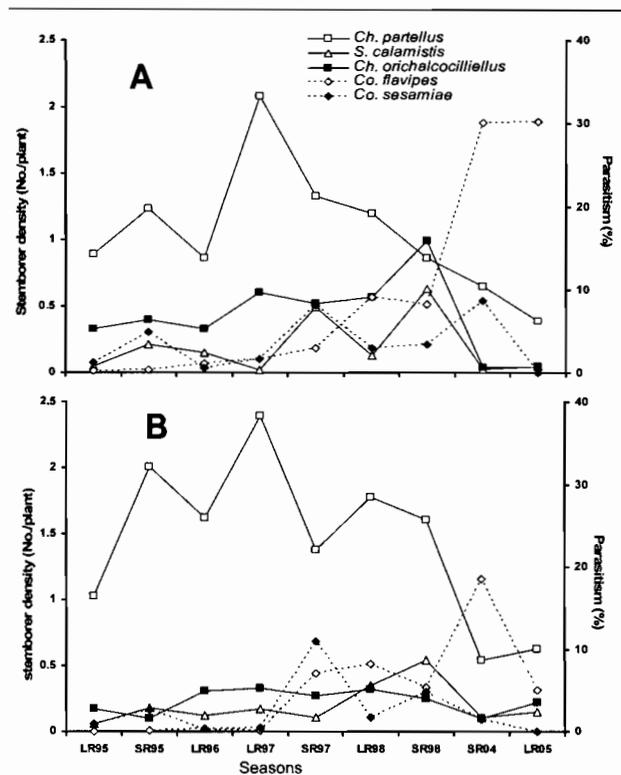

Figure 2
Seasonal population dynamics of stemborer and parasitism in (A) north and (B) south coast. X-axis is the season of the year (e.g. LR95 and SR95 refers to long and short rainy season of 1995, respectively).
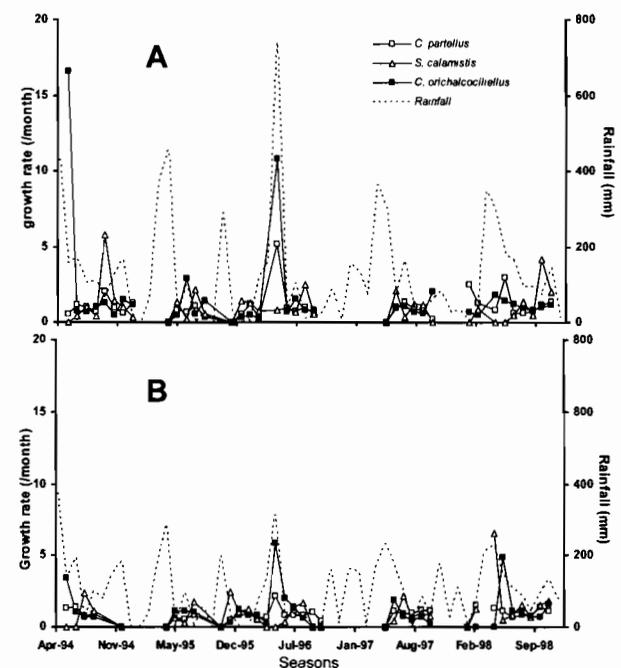

Figure 3
Seasonal population growth rate of stemborer with rainfall in mm in (A) north and (B) south coast. X-axis is the month of the year.

model, with a significant correlation with rainfall at *t-2*. For *S. calamistis* population, it was a first-order AR model and there was no correlation with rainfall.

In the south coast, population growth rates of all three stemborer species had a 1st and 2nd -order time lags indicating that all species were affected by parasitoids, and all were correlated with rainfall at time *t*.

Spatial synchrony of stemborer populations

Figures 4a & b show the spatial non-parametric correlation function (SNCF) of *Ch. partellus*. Its average regional synchrony was 0.36 before 1997, and the confidence intervals for the mean correlation coefficient were mostly positive (fig. 4a), indicating that the dynamics of the host was regionally affected by environmental factors. After 1996, this value was reduced to 0.21 and the correlation between

neighboring *Ch. partellus* populations decreased with distance (fig. 4b) indicating that the parasitoid reduced the spatial synchrony of the population. The U-shaped curve means a second order of the spatial relationship as a result of probably parasitism.

Before 1997, the spatial correlation of *S. calamistis* showed an average synchrony value of 0.27 across the region, indicating that the synchrony was driven by climatic factors, and the population was stable (fig. 4c). After 1996, the regional synchrony was also reduced to 0.20 by biological control (fig. 4d). By contrast, the spatial synchrony of *Ch. orichalcociliellus* increased from 0.15 to 0.22, after 1996 (fig. 4e & f), and the population varied from a non-linear curve to a stabilizing spatial distribution, which indicates that after *Co. flavipes* was introduced in the area, spatial synchrony of *Ch. orichalcociliellus* was more affect by regional climates than its parasitism (fig. 4e). Among the three stemborer species, *Ch. partellus* populations had the highest regional synchrony before 1997, but afterwards, the average regional synchrony of the three species was similar.

Spatial cross-correlation of stemborer and parasitoid populations

Among all spatial non-parametric cross-correlations (SNCCF) between stemborer and *Co. flavipes*, the correlation between *Ch. orichalcociliellus* and *Co. flavipes* was the highest at 0.15 (fig. 5e); it decreased with distance after 80 km. The correlations of *Ch. partellus* and *Ch. orichalcociliellus* with *Co. flavipes* were similar (fig. 5a). However, the former correlation had the lowest value of 0.04. The correlation of *S. calamistis* with *Co. flavipes* showed that there was a periodic-distance distribution of their host-parasitoid interaction (fig. 5c).

The spatial regional synchrony of all three stemborer populations with *Co. sesamiae* was similar (figs. 5b, d, f). The highest value of 0.11 was obtained for *S. calamistis* with *Co. sesamiae*. The spatial synchrony relationship of *Co. sesamiae* with *Ch. partellus* and *S. calamistis* was higher than that of *Co. flavipes*, except for *Ch. orichalcociliellus*. The positive confidence interval between the host and the parasitoid suggests a synchrony between the pest and its natural enemy.

Figure 4
Spatial correlation of stemborer with distance: (a) and (b) are *Ch. partellus* before and after the end of 1996; (c) and (d) are *S. calamistis* (e) and (f) are *Ch. orichalcociliellus*. X-axis is the distance between sampling sites (km), y-axis is the spatial correlation coefficient of the stemborer species. The middle solid line of the curves is the correlation coefficient, and the upper and lower superimposed dashed lines represented 95% of confidence interval.

Discussion

Even though the time series data set of this study was not very large, it included three stemborer and two parasitoid species as well as rainfall data, which could explore the mechanism driving this complex system. Thereby, the intra- and inter-specific competition

among the exotic and indigenous stemborer species and parasitoids would affect their spatial and temporal dynamics. In the past 40 years, *Ch. orichalcociliellus* was increasingly displaced by *Ch. partellus*. Its percentage on total stemborers was reduced from 66% to 14%, while *Ch. partellus* increased from 28.5% to 87% (Mathez 1972; Zhou *et al.* 2001b). The present findings showed that the regional synchrony of *Ch. orichalcociliellus* had increased from before 1997, suggesting that the population of *Ch. orichalcociliellus* coexists with *Ch. partellus* in a stable manner. *Co. flavipes* and *Co. sesamiae* accounted for 83% of the parasitoid species on cereals in coastal Kenya with *Co. flavipes* as the dominant species (Zhou *et al.* 2003). Will *Co. flavipes* finally displace *Co. sesamiae*? Our results showed that the cross-correlation coefficients of stemborers with *Co. sesamiae* were almost always positive, which means that it would not get extinct as also hypothesized by Sallam *et al.* (2001).

Chilo spp. larvae enter the diapause under unfavorable climatic conditions, i.e. the dry season and the dry spell between the two rainy seasons (Ofomata *et al.* 1999). The starting of the first rainy season is critical for both stemborer emergence from diapause and growth of its host plants, maize mainly. Therefore, the duration of the short dry spell between long and short rain season is assumed to be important for population growth of borers. In addition, drought stress affects nitrogen levels in the plant (Janssen 1993) which, in turn, influences survival of stemborers feeding on plant and the performance of the parasitoid (Jiang & Schulthess 2005). In general and as also shown for *S. calamistis* and *E. saccharina* in West Africa, borer densities should increase from the long to the short rainy season (Schulthess *et al.* 1997) unless they are controlled by natural enemies as reported for *B. fusca* by Ndemah *et al.* (2003). In the present study, borer densities, and especially those of indigenous borer species, were lower during the long than the short rainy seasons indicating that, as in West Africa, borer densities increase in course of the two seasons. Moreover, the 2nd order of rainfall of the time series correlation analysis in the north coast on *Chilo* spp. indicated that its effect on the population dynamics was indirect through density-dependent effects on the population. By contrast, in the south coast, all stemborer species were affected by a first-order feedback loop and the positive effect of rainfall on *Chilo* spp. suggesting that the host plants (wild and cultivated) were the limiting factor. Therefore, it can be concluded that in the north coast, stemborers had a higher physiological adaptation to

climatic variations, while in the south coast they were more dependent on the host plants; it appears that host plant abundance is higher in the south than north coast. Stenseth *et al.* (2002) also stressed the importance to study the relationship between ecological pattern (e.g. density-dependence or independence) with non- or interactive effect of climates.

Different population dynamics patterns may be produced by difference in the feedback structures (Berryman 2001; Lima *et al.* 2002). The autoregressive model analysis showed that in the north coast, *S. calamistis* consisted of an auto-correlated population not affected by rainfall effect but rather by intra-specific competition. *Chilo* spp. had a strong 2nd order of the time series correlation which indicates a trophic level feedback from natural enemies. In the south coast, all stemborer species were affected by a 2nd order feedback from parasitism, and especially by *Cotesia* spp.,
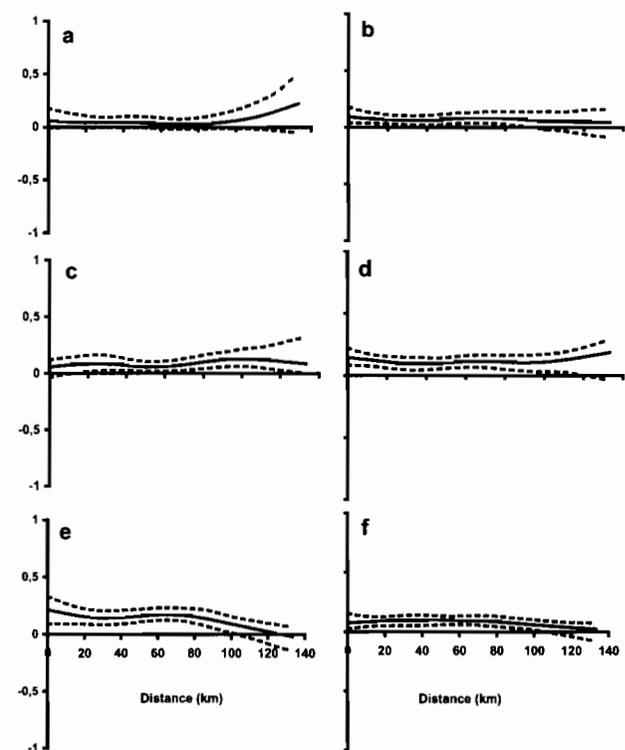

Figure 5
Cross-correlation coefficient between stemborer and parasitoid with distance, (a) *Cotesia flavipes* and (b) *Co. sesamiae* with *Ch. partellus*, (c) and (d) are *Co. flavipes* and *Co. sesamiae* with *S. calamistis*; and (e) and (f) are *Co. flavipes* and *Co. sesamiae* with *Ch. orichalcociliellus*. X-axis is the distance between sampling sites (km), y-axis is the spatial cross-correlation coefficient between stemborer and parasitoid species. The middle solid line of the curves is the correlation coefficient, and the upper and lower superimposed dashed lines represented 95% of confidence interval.

which had feedback via density-dependence. Zhou *et al.* (2001a) used a ratio-dependent host-parasitoid interaction model to study the effect of parasitoid on stemborer population dynamics in the north and south coast of Kenya, but without considering climatic factor and only the first time lag of the stemborer auto-regression model was assumed.

Research on the ecology of infectious diseases (Bolker & Grenfell 1996) showed that control interventions, such as vaccinations, could lead to asynchrony or synchrony depending on details of their natural history (Bjørnstad et al. 1999). Classical biological control used in the integrated pest management acts in a similar manner. Our study showed that introduction of *Co. flavipes* reduced the average spatial synchrony of *Ch. partellus* and *S. calamistis*, and increased the synchrony of *Ch. orichalcociliellus*. The success of biological control can be related to biological, physiological, and/or environmental limitations on the life cycle of parasitoids. Asynchrony may occur if parasitoids introduced into new areas for which they are imperfectly adapted (Goldson & Emberson 1980; Goldson & McNeil 1992; Godfray *et al.* 1994), or the host refugee makes the parasitoid difficult to find the host. After the introduction of *Co. flavipes*, *Ch. partellus* had a closer relationship with its neighboring populations indicating that the local dispersal became more important than environmental factors. Since *Ch. orichalcociliellus* was found to have a strong niche overlap with *Ch. partellus* but with a wider host range than *Ch. partellus* (Ofomata *et al.* 2000), the higher parasitism of *Co. flavipes* on *Ch. partellus* could cause a reduction in the inter-specific competition between *Ch. orichalcociliellus* and *Ch. partellus* and an increase in the spatial synchrony. The native stemborer, *S. calamistis* seems to have a strong spatial correlationship with the native parasitoid *Co. sesamiae*, and it was not affected by the introduction of *Co. flavipes*.

Differences in the relative and absolute mobility of interacting species affect the spatial structure of the spatial pattern of host and parasitoid, causing e.g. spatial chaos, spiral waves or crystal lattices (Hassell *et al.* 1991; Comins *et al.* 1992), and different cross-correlation spatial synchrony (Bjørnstad & Bascompte 2001). Zhou & Overholt (2001) studied the spatial-temporal population dynamics and distribution of *Co. flavipes* in Kenya, and found that the spatial-temporal variation of the population was large, and *Co. flavipes* dispersed from coastal area to the central region. However, the degree of mobility and dispersal of stemborers and parasitoids are unknown. In our study, the positive cross-correlation of stemborers and parasitoids points to density-dependence

between the parasitoid and its hosts. Although parasitism of *Co. flavipes* was higher than that of *Co. sesamiae*, the cross-correlations of *Ch. partellus* and *S. calamistis* with *Co. sesamiae* were higher than their relationship with *Co. flavipes*. This indicates that their relationship with the indigenous species is more affected by environmental factors than the exotic parasitoid, indicating that the parasitoid is still dispersing and has not yet reached an equilibrium with its hosts. *Ch. orichalcociliellus* had a stronger relationship with *Co. flavipes* than the other two species, underlining its potential as a host for *Co. flavipes*.

The spatial-temporal synchrony of multi-species stemborer was not only affected by the introduction of biological control agent, but also interacted with the climatic condition and their inter-specific competition. Large-scale studies on the population dynamics integrated with spatial habitat distribution of stemborers would be necessary in the future to study the mechanism of the synchrony.

References

Berryman A.A. 2001. Functional web analysis: detecting the structure of population dynamics from multi-species time series. *Basic and Applied Ecology* 2: 311-321.

Bjørnstad O.N., Bascompte J. 2001. Synchrony and second-order spatial correlation in host-parasitoid systems. *Journal of Animal Ecology* 70: 924-933.

Bjørnstad O.N., Bolker B. 2000. Canonical functions for dispersal-induced synchrony. *Proceedings of the Royal Society of London Series B* 267: 1787-1794.

Bjørnstad O.N., Falck W. 2001. Non parametric spatial covariance functions: estimation and testing. *Environmental and Ecological Statistics* 8: 53-70.

Bjørnstad O.N., Ims R.A., Lambin X. 1999. Spatial population dynamics: analyzing patterns and processes of population synchrony. *Trends in Ecology and Evolution* 14: 427-433.

Bjørnstad O.N., Peltonen M., Liebhold A.M., Baltensweiler W. 2002. Waves of larch budmoth outbreaks in the European alps. *Science* 298: 1020-1023.

Bolker B.M., Grenfell B.T. 1996. Impact of vaccination on the spatial correlatin and persistence of measles dynamics. *Proceedings of the National Academy of Science* 93: 12648-12653.

Burnham K.P., Anderson D.R. 1998. *Model Selection and Interferce: A Pratical Information Theoretic Approach.* Spring-Verlag, New York.

Cattadori I.M., Haydon D.T., Hudson P.J. 2005. Parasites and climate synchronize red grouse poulations. *Nature* 433: 737-741.

Comins H.N., Hassell M.P., May R.M. 1992. The spatial dynamics of host-parasitoid systems. *Journal of Animal Ecology* 61: 735-748.

Godfray H.C.J., Hassell M.P., Holt R.D. 1994. The population dynamic consequences of phonological asynchrony between parasitoids and their hosts. *Journal of Animal Ecology* 63: 1-10.

Goldson S.L., Emberson R.M. 1980. Relict diapause in an introduced weevil in New Zealand. *Nature* 286: 489-490.

Goldson S.L., McNeill M.R. 1992. Variation in the critical photoperiod for diapause induction in *Microclonus hyperodae*, a parasitoid of Argentine stem weevil. *Proceedings 45th New Zealand Plant Protection Conference* 205-209.

Hassell M.P., Comins H.N., May R.M. 1991. Spatial structure and chaos in insect population dynamics. *Nature* 353: 255-258.

Hudson P.J., Cattadori I.M. 1999. The Moran effect: a cause of population synchrony. *Trends in Ecology and Evolution* 14: 1-2.

Janssen J.A.M. 1993. Soil nutrient availability in a primary outbreak area of the African armyworm, *Spodoptera exempta* (Lepidoptera: Noctuidae) in relation to drought intensity and outbreak development in Kenya. *Bulletin of Entomological Research* 83: 579-593.

Jiang N., Schulthess F. 2005. The effect of nitrogen fertilizer application to maize and sorghum on the bionomics of *Chilo partellus* (Lepidoptera: Crambidae) and the performance of its larval parasitoid *Cotesia flavipes* (Hymenoptera:Braconidae). *Bulletin of Entomological Research* 95: 495-504.

Lima M., Stenseth N.C., Jaksic F.M. 2002. Food web structure and climate effects on the dynamics of small mammals and owls in semi-arid Chile. *Ecology Letters* 5: 273-284.

Mathez F.C. 1972. *Chilo partellus* Swinh., *C. orichalcociliella* Strand (Lep., Crambidae) and *Sesamia calamistis* Hmps. (Lep., Noctuidae) on maize in the coast province, Kenya. *Bulletin de la Société Entomologique Suisse* 45: 267-289.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Poehling M., Cardwell K.F. 2003. Factors affecting infestations of the stalk borer *Busseola fusca* (Fuller) on maize in the forest Zone of Cameroon with special reference to scelionid egg parasitoids. *Environmental Entomology* 32: 61-70.

Ofomata V.C., Overholt W.A., Egwuatu R.I. 1999. Diapause termination of *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* Strand (Lepidoptera: Pyralidae). *Insect Science and its Application* 19: 187-191.

Ofomata V.C., Overholt W.A., Lux S.A., van Huis A., Egwuatu R.I. 2000. Comparative studies on the fecundity, egg survival, larval feeding, and development of *Chilo partellus* and *Chilo orichalcociliellus* (Lepidoptera: Crambidae) on five grasses. *Annals of the Entomological Society of America* 93: 492-499.

Overholt W.A., Ngi-Song A.J., Kimani S.W., Mbapila J., Lammers P.M., Kioko E. 1994a. Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in Africa. *Biocontrol News Information* 15: 19-24.

Overholt W.A., Ngi-Song A.J., Omwega C.O., Kimani-Njogu S.W., Mbapila J., Sallam M.N., Ofomata V. 1997. A review of the introduction and establishment of *Cotesia flavipes* Cameron in East Africa for biological control of cereal stemborers. *Insect Science and Its Application* 17: 79-88.

Overholt W.A., Ogedah K., Lammers P.M. 1994b. Distribution and sampling of *Chilo partellus* (Lepidoptera: Pyralidae) in maize and sorghum on the Kenya coast. *Bulletin of Entomological Research* 84: 367-378.

Peltonen M., Liebhold A.M., Bjørnstad O.N., Williams D.W. 2002. Spatial synchrony in forest insect outbreaks: roles of regional stochasticity and dispersal. *Ecology* 83: 3120-3129.

Ranta E., Kaitala V., Lindström J., Lindén H. 1995. Synchrony in population dynamics. *Proceedings of the Royal Society of London B* 262: 113-118.

Sallam M.N., Overholt W.A., Kairu E. 1999. Comparative evaluation of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae) for the management of *Chilo partellus* (Lepidotera: Pyralidae) in Kenya. *Bulletin of Entomological Research* 89: 185-191.

Sallam M.N., Overholt W.A., Kairu E., Zhou G. 2001. Will *Cotesia flavipes* drive its African homologue, *Cotesia sesamiae*, to extinction? *Insect Science and its Application* 21: 297-302.

SAS Institute 2000. *Users' guide: Statistics, release 8.0 ed.* SAS Institute, Cary, NC.

Schulthess F., Bosque-Pérez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Goergen G. 1997. Exchange of natural enemies of lepidopteran cereal stemborers between African regions. *Insect Science and its Application* 17: 97-108.

Stenseth N.C., Mysterud A., Ottersen G., Hurrell J.W., Chan K.-S., Lima M. 2002. Ecological effects of climate fluctuations. *Science* 297: 1292-1296.

Tams W.H.T. 1932. New species of African Heterocera. *Entomologist* 65: 1241-1249.

Tobin P.C., Bjørnstad O.N. 2003. Spatial dynamics and cross-correlation in a transient predator-prey system. *Journal of Animal Ecology* 72: 460-467.

Zhou G., Baumgartner J., Overholt W.A. 2001a. Impact of an exotic parasitoid on stemborer (Lepidoptera) populations dynamics in Kenya. *Ecological Applications* 11: 1554-1562.

Zhou G., Overholt W.A. 2001. Spatial-temporal population dynamics of *Cotesia flavipes* (Hymenoptera: Braconidae) in Kenya. *Environmental Entomology* 30: 869-876.

Zhou G., Overholt W.A., Kimani-Njogu S.W. 2003. Species richness, parasitism and trophic relationships in an assemblage of parasitoids attacking maize stem borers in coastal Kenya. *Ecological Entomology* 28: 109-118.

Zhou G., Overholt W.A., Mochiah M.B. 2001b. Changes in the distribution of lepidopteran maize stemborers in Kenya from the 1950s to 1990s. *Insect Science and its Application* 21: 395-402.

Distribution and relative importance of cereal stem borers and their natural enemies in the semi-arid and cool-wet ecozones of the Amhara State of Ethiopia

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Abstract. The distribution and relative importance of lepidopteran and coleopteran stem borers and their natural enemies on maize and sorghum were studied in cereal growing zones of the Amhara State of Ethiopia from 2003 to 2004. Sorghum is the major crop in semi-arid eastern and maize in the cool-wet western zones of the Amhara state. Four administrative zones, 10 districts and 88 localities in the semi-arid ecozone (SAE) and four zones, 19 districts and 71 localities in the cool-wet ecozone (CWE) were chosen for the study. In SAE, the species composition was 91% *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), 8% *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and 1% *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). In the CWE, maize and sorghum are grown in different ecozones and thus *B. fusca* was the dominant species on sorghum, whereas 61% *B. fusca* and 39% *S. calamistis* were recorded on maize. Borer density generally increased with crop growth stage. *C. partellus* parasitism by *C. flavipes* Cameron (Hymenoptera: Braconidae), which occurred only in SAE, varied among districts ranging from 5% to 39%. In the CWE, unidentified nematodes parasitized medium-sized *B. fusca* larvae during the wet months. Population of native parasitoids was very low. The coleopteran borer, *Rhynchaenus niger* (Horn) (Coleoptera: Rhynchophoridae), attacked sorghum plants in both regions. Sorghum yields were negatively related to plant damage variables and positively to larval parasitism and plant growth variables. On maize, plant damage was too low to affect yields. Taylor's power law indicated aggregated distribution for *C. partellus* and *B. fusca* larvae and pupae combined.

Résumé. Distribution et importance relative des foreurs de graminées et de leurs ennemis naturels dans les zones semi-arides et plus humides et froides de l'état d'Amhara en Ethiopie. La distribution et l'importance relative des lépidoptères et coléoptères foreurs ainsi que de leurs ennemis naturels ont été étudiées de 2003 à 2004 dans les zones de culture du maïs et du sorgho de l'état d'Amhara en Ethiopie. Le sorgho est principalement cultivé dans les zones semi-arides de l'Est de l'état, alors que le maïs est plutôt cultivé dans des zones froides et humides à l'Ouest. Quatre zones administratives ont été choisies pour cette étude dans les zones semi-arides comprenant 10 districts et 88 localités; et quatre autres zones administratives comprenant 19 districts et 71 localités dans les zones froides et humides. Dans les zones semi-arides, parmi les espèces de lépidoptères collectés, 91% a été représenté par *Chilo partellus* (Swinhoe) (Lepidoptera : Crambidae), 8% par *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae) et 1% par *Sesamia calamistis* Hampson (Lepidoptera : Noctuidae). Dans les zones froides et humides, le maïs et le sorgho ne sont pas cultivés ensemble, mais dans différentes zones écologiques. De ce fait, *B. fusca* était l'espèce de lépidoptère foreur dominante sur sorgho, alors qu'elle ne représentait que 61% sur maïs où *S. calamistis* était aussi présent pour 39%. La densité de lépidoptères foreurs a augmenté généralement avec le stade de développement des plantes. Le taux de parasitisme de *C. partellus* par *Cotesia flavipes* Cameron (Hymenoptera : Braconidae), qui est présent seulement dans les zones semi-arides, variait selon les districts de 5% à 39%. Dans les zones froides et humides, une espèce de nématode non identifiée attaquait les larves de *B. fusca* de taille moyenne pendant les mois les plus humides. La population des parasitoïdes autochtones de cette zone était très faible. Le coléoptère foreur, *Rhynchaenus niger* (Horn) (Coleoptera : Rhynchophoridae), infestait le sorgho dans les deux types de régions. Le rendement en sorgho était négativement corrélé aux paramètres liés aux dommages de la plante et positivement au parasitisme larvaire et aux variables liées à la croissance de la plante. Sur maïs, les dommages de la plante étaient trop faibles pour en affecter son rendement. La loi de Taylor a indiqué une distribution d'agrégation pour les larves et les chrysalides de *C. partellus* et *B. fusca*.

Keywords: Stem borer, parasitoids, importance, distribution, Ethiopia.

In Africa, maize and sorghum are major staples with ca 42 and 20 million tons produced, respectively, in 2002 (FAO 2003). In Ethiopia, these crops are grown on 2.4 million hectares contributing about 41% of the country's annual grain production (CSA 2000; CACC 2003). Maize is one of the major staple food crops, 90% of which is used mainly for human consumption (Ferdu *et al.* 2001). It thrives well in cool and wet intermediate altitudes (1500-2000 m above sea level [m asl]), while sorghum is the dominant crop in the lowlands (< 1500 m asl). The major pests of field-grown maize are the crambid *Chilo partellus* (Swinhoe 1885), the noctuids *Busseola fusca* (Fuller 1901) and *Sesamia calamistis* Hampson 1910 (Assefa 1985), and various species of termites (*Macrotermes* and *Microtermes* species). In addition to those species, *Sesamia nonagrioides botanephaga* (Lefèbvre 1827), *Rhynchaenus niger* (Horn 1873) (Coleoptera: Curculionidae), *Pissodes dubius* (Strom 1783) (Coleoptera: Curculionidae) have been recorded from sorghum (Emana 2002). Recently, the exotic braconid parasitoid *Cotesia flavipes* Cameron 1891 was found parasitizing *C. partellus* larvae in some parts of Ethiopia (Emana 2002; Emana *et al.* 2003). This parasitoid was introduced into Kenya in 1993 by the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, as part of a classical biological control program now encompassing eleven countries in East and Southern Africa (Overholt *et al.* 1994a). In coastal Kenya, it has reduced *C. partellus* densities by 60% and increased yields by 10-15% (Overholt *et al.* 1997; Zhou *et al.* 2001). It is believed that it has invaded Ethiopia probably from Somalia where it was introduced in 1997 (C. Omwega, ICIPE, Kenya, pers. comm.). Molecular analysis of the Ethiopian and Kenyan *C. flavipes* populations has revealed 100% similarity (Yoseph Assefa, KwaZulu-Natal University, South Africa, *pers. comm.*).

Reported crop losses in Ethiopia by *C. partellus* and *B. fusca* range from 15 to 100% (Assefa 1989; Tadesse 1989; Gashawbeza & Melaku 1996; Emana 1998). Although the Amhara State is contributing one-third of both the area and production of the country's maize and sorghum grain (BOA 1999; CACC 2003), information about the species composition and pest status of stem borers and their natural enemies in the region is scanty (Shegaw *et al.* 1999). Borers are believed to have aggravated recurrent famines especially in semi-arid eastern Amhara (BOA 1999; Emana 2002). Information about the relative importance of a key pest is a prerequisite for priority setting in pest management (Ndemah *et al.* 2001a). Thus, the objective of the present study was to assess the distribution and importance of stem borer species and to establish a catalogue of natural enemies in the Amhara State.

Materials and methods

Study area

The study was conducted in 2003 and 2004 in the Amhara National Regional State (ANRS), which is located in north-western, north-eastern and central parts of Ethiopia. In this study, the Amhara State is divided into two ecologically distinct parts, i.e., semi-arid eastern and cool-wet western Amhara. The State is situated between 8° 45' N to 13° 45' N latitude and 35° 46' E to 40° 25' E longitude and has an area of 170,000 km² (PEDB 1999). Rainfall gradually increases from 700 mm in the semi-arid to over 2000 mm in the cool-wet western Amhara. In the cool-wet ecozone, there is one effective rainy season that lasts for three to six months (June to November), its intensity and duration increasing westward. However, there is a short rainy period, lasting about a month, around April, which is not sufficient to grow crops, but it is used for land preparation and to plant long maturing sorghum varieties in some highlands of East Gojam.

The semi-arid eastern part, however, is sufficiently bimodal as it also receives some rains from March to May (short rains) due to easterly winds, in addition to the main rainy season of mid-June to September. The study areas in the semi-arid region of the Amhara state, which are planted mainly to sorghum, lay between 1200 to 1985 m asl and the cool-wet western region, mostly planted to maize, lay between 1300 and 2600 m asl.

Sampling procedures

In the cool-wet ecozone, four administrative zones, i.e., East Gojam, West Gojam (maize belt of the State), South Gondar and North Gondar, and in the semi-arid ecozone four sorghum producing zones, i.e., North Shoa, Oromiya, South Wolo and North Wolo of the Amhara State were studied. A total of 159 localities (fields) were surveyed (fig. 1). In the semi-arid eastern ecozone, 88 localities within 10 districts and 4 zones, and in the cool-wet ecozone, 71 localities within 19 districts and 4 zones were sampled. The number of localities and the distance between them varied between zones depending on the scale of cereal production. In areas of continuous production, fields were selected at 10-km intervals. At each locality, more accessible fields close to the road were sampled. The latitude, longitude, and elevation of each study site were determined with a Global Positioning System (GPS).

In both regions, fields were sampled at various growth stages of the crops. These include the seedling, knee height, flag leaf, tasseling, grain filling and harvest stages. The study was mostly conducted during the main growing season. Length of time between stages varied with crop variety and ecozone. In the semi-arid region, both late, which need two consecutive seasons, i.e. about 9-months, and early, 4 to 5-month, maturing sorghum varieties were planted; maize is always early maturing in this region. The cool-wet ecozone received rains one month earlier than the semi-arid ecozone but crops in the latter region grew faster due to higher temperature and matured almost at the same time as in the cool-wet region. Number of localities surveyed at each growth stage varied from 31 to 57 in the semi-arid, and 29 to 45 in the cool-wet region. Much of the semi-arid eastern Amhara is planted with sorghum, whereby late

maturing varieties, which grow as tall as 4m, are planted during the short rains in April and are harvested in December. Different sorghum varieties were identified based on the duration to maturity. Long maturing refers to 8-9 month, medium refers to 6-7 month and early for 4-5 month varieties. Maize is also grown in semi-arid eastern Amhara, especially during the short rains, i.e., from around February to May, and harvested before the main rainy season, i.e., June to September. In the semi-arid region, the field size varied from less than a quarter of a hectare

in hilly terraces to tens of hectares grown in extensive vertic plains. In this same region, some 60% of farmers intercrop sorghum with beans or sesame. In the cool-wet ecozone, on the other hand, field sizes varied from less than a hectare to about two hectares and most farmers traditionally intercrop maize with mustard and faba bean on a wide variety of soil types.

Destructive sampling procedure was used. Each field was divided into 4 sections, from which 3×3 m² quadrants were sampled. On each quadrant, total plant density, number of
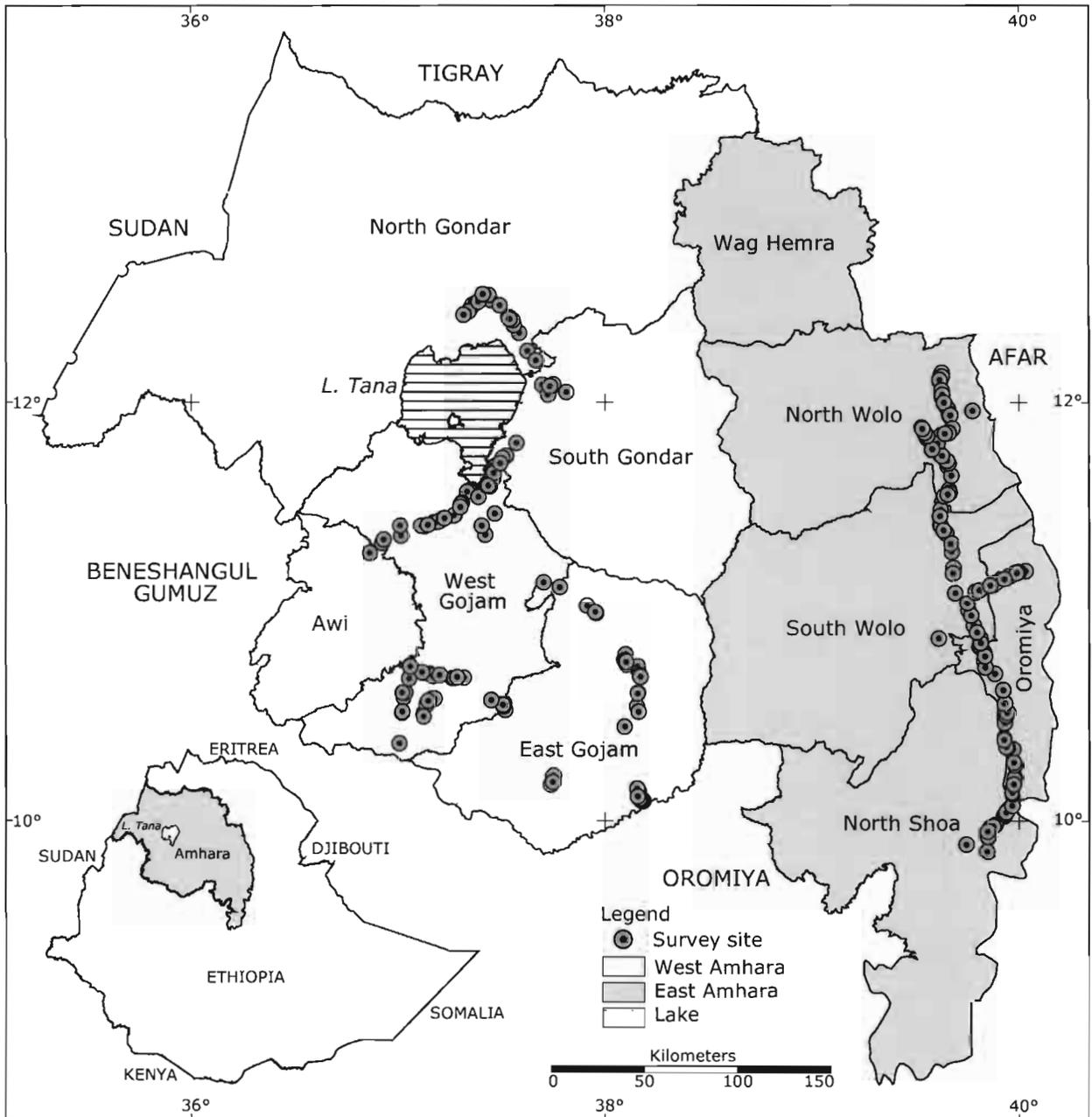

Figure 1
Survey localities in the Amhara state, 2003 and 2004 (dotted circles indicate survey localities, West Amhara is the cool-wet and East Amhara the semi-arid ecozones, and L Tana indicates Lake Tana).

plants infested (dead-heart and leaf damage) and un-infested were recorded. Five plants were then randomly selected per quadrant and dissected to determine the number of larvae and pupae as well as of natural enemies. Plant growth stage, plant height, basal stem diameter, number of internodes (damaged and undamaged), length of stem tunnelling, number of holes, number of borers, *Cotesia* spp. cocoon masses and other parasitoids and earwigs were recorded. At harvest peduncle for sorghum and cob for maize damage per plant, and grain yield from a 16 m² area per field were estimated. Peduncle damage was assessed by observing the peduncle. It could be either damaged or undamaged. This damage symptom is common observation in the area, even when other parts of the plant are not attacked. Cob damage was estimated by counting the number of grain bearing rows in a cob. If one row of 10 in a cob was fully infested, we took that as 10% infested, etc. A sample of the grain was taken and its moisture content determined using a moisture tester and the yield was then adjusted at 14% moisture content for both crops.

Borer larvae were kept in 3 × 1 inch vials and reared on pieces of sorghum or maize stem until adult moth or parasitoid emergence. The parasitoids were identified at the International Center of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya.

Statistical analysis

Analyses of variance (ANOVA) were conducted using the mixed model procedure (SAS 1999-2000) to determine variation in borer density, natural enemies (% larval parasitism and of earwig density), plant damage variables and yield between years, regions, zones, and crop growth stages. Locality, district, year x zone x district x locality constituted random effects, while year, zone, crop growth stage and variety constituted fixed factors. Localities were nested within districts, districts within zones, zones within years. The percentage variance estimates for the random effects were calculated, and when the mean estimates of fixed factors were significant, they were separated using Tukey-Kramer test.

Correlation analyses were conducted to assess interactions between plant growth, plant damage, borer density, percent parasitism, altitude, and plant growth stage using mean data for each locality.

Stepwise multiple regressions were computed to evaluate the effects of plant growth parameters, borer damage and parasitism to sorghum and maize yields (SAS 1999-2000).

Taylor's (1961) power law was used to describe the dispersion of borer larvae and pupae at different growth stages of the crop. The law postulates a consistent relationship for a particular species between variance S² and the mean X:

$$S^2 = aX^b \qquad (1)$$

where b is a measure of dispersion of the species, with $b>1$ indicating an aggregated distribution, $b = 1$ randomness, and $b < 1$ regular distribution, while a is considered a mere scalar factor without biological meaning. These coefficients were computed by regressing the natural logarithm of the between plant variance (lnS^2) against the natural logarithm of mean density (ln X), for each crop species, crop growth stage, region, or sampling occasion. Slope values across crop growth stages of a particular borer species were compared by using the PROC GLM and also with 1 using the PROC REG procedures of SAS.

When necessary, percentage data were arcsine and counts log (x+1) transformed to stabilize the variance. The significance level was set at $P = 0.05$.

Results

Seasonal and regional variation in abundance of stem borers and their natural enemies

Stem borers

Across sampling periods, 12,500 borers were collected in the two agroecozones investigated. Borer density and species composition varied with region (semi-arid eastern and the cool-wet western Amhara), administrative zone, year, crop phenology, crop type and variety (tab. 1). Of the total borers collected, 75.4% were found in the semi-arid eastern (SAE) and 24.6% in the cool-wet ecozone (CWE) regions. Furthermore, 62.2% were *C. partellus*, 33.7% *B. fusca* and 4.1% *S. calamistis*. Again, 60.2% of borers were *C. partellus* borers found on sorghum and 2% on maize; 23.2% were *B. fusca* borers on sorghum and 8% on maize; and, some 1% *S. calamistis* borers on sorghum and 3.2% on maize. In addition, 154 *R. niger* stemboring grubs and adults were collected from inside the dissected stems. In the cool-wet region, where maize was the major crop, *B. fusca* and *S. calamistis* were of similar importance, especially in areas near Lake Tana, while on sorghum, *B. fusca* was dominant and *S. calamistis* was rare. In the semi-arid region, where sorghum was the major crop, the predominant species was *C. partellus* followed by *B. fusca* and *S. calamistis* (tab. 1).

The species composition was similar during both years. In 2004, in semi-arid region, on sorghum, the composition was 91% *C. partellus*, 8% *B. fusca* and 1% *S. calamistis*.

For total borer density on sorghum, random effects (Locality x District x Zone x Year, hereafter referred to as L x D x Z x Y) contributed 34% of the variation. On both plant species most of the variation in borer densities was due to residual, i.e., between plant variation followed by L x D x Z x Y and Z x Y (tab.1).

In the cool-wet region, on sorghum, it was solidly *B. fusca*, whereas on maize 61% *B. fusca* and 39% *S. calamistis* were recorded; at tasseling stage, however, 91% of the borers were *S. calamistis*.

R. niger was found on sorghum in both regions. Densities were generally low but they were higher in semi-arid eastern Amhara. Total borer densities on sorghum tended to be higher in eastern (semi-arid ecozone) than the cool-wet western Amhara and they were higher in the 2003 than the 2004 season (tab. 1). On both sorghum and maize, borer densities increased from the seedling to the grain filling stage and then decreased at harvest. As it can be seen on sorghum in table 1, overall densities of *C. partellus* in the Amhara state were 2–4 times higher than those

of *B. fusca*. In most study areas, *S. calamistis* was minor on sorghum, but surprisingly it was the only species on maize in West Gojam and South Gondar zones bordering Lake Tana (tab. 1; fig. 1). This area specifically covers the plains in the southeastern parts of the lake, which crosses the two neighboring zones. Its importance declines as we move away from the lake area. In the semi-arid region, total borer density and *Ch. partellus* steadily decreased

northwards from North Shoa to North Wolo zones (tab.1; fig. 1). Unlike *R. niger*, lepidopterous borer density was significantly higher on the traditional long maturing sorghum varieties than medium and early maturing ones (tab.1).

Natural enemies

Across all sampling periods, 1879 cocoon masses were collected from inside the dissected stems. C.

Table 1. Effects of year, location and crop growth stage on abundance of borers per plant on sorghum and maize in the semi-arid and cool-wet ecozones of Amhara state, Ethiopia, in 2003 and 2004.

	Sorghum	Maize	Sorghum	Sorghum			Maize	
	Total lepidopterous borers/ plant		R. niger/ plant	C. partellus	B. fusca	S. calamistis	B. fusca	S. calamistis
Random effects				% Variation explained				
Locality (D x Z x Y)*	34.0	12.9	21.1	32.6	23.5	24.7	10.4	6.1
Zone (year)	11.3	14.0	43.0	17.0	1.3	0.5	9.1	4.5
District	0.0	2.8	0.9	0.0	16.2	0.1	7.1	0.0
Residual	54.7	70.3	35.0	50.4	59.0	74.7	73.4	89.4
Fixed effects				Least square means (±SE)				
Year								
2003	2.4±0.46a	0.56±0.30a	0.091±0.01a	1.86±0.34a	0.50±0.11a	0.03±0.01a	0.37±0.31a	0.09±0.06a
2004	1.1±0.43b	0.01±0.33b	0.001±0.01b	0.83±0.31b	0.28±0.10b	0.01±0.01b	0.01±0.33b	0.07±0.05a
F value	15.90	13.29	89.98	12.10	6.54	9.82	16.62	0.09
P value	<.0001	0.0007	<.0001	0.0006	0.0114	0.0020	0.0002	0.7666
Administrative zone (No. localities) **								
East Gojam (20)	1.2±0.8b	0.67±0.3a	0.001±0.01c	DE	0.93±0.16a	0.0030±0.01b	0.52±0.32	0.09±0.05
West Gojam (22)	0.0±2.3c	0.28±0.3a	0.020±0.02bc	DE	0.00±0.55b	0.0003±0.06ab	0.05±0.24	0.13±0.04
South Gondar (16)	1.4±1.4b	0.01±0.6a	0.001±0.02c	DE	0.43±0.31a	0.0100±0.02ab	0.10±0.32	0.11±0.08
North Gondar (20)	1.8±0.8b	0.00±0.9a	0.001±0.01c	DE	0.93±0.18a	0.0100±0.01ab	0.15±0.12	0.00±0.16
North Shoa (15)	3.2±1.1a	-	0.080±0.02b	2.94±0.49a	0.23±0.21a	0.0050±0.01b	-	-
Oromiya (24)	2.9±0.7a	-	0.070±0.01b	2.64±0.35a	0.05±0.15b	0.0060±0.01b	-	-
South Wolo (22)	2.0±0.7b	-	0.131±0.01a	1.78±0.37b	0.25±0.15a	0.0500±0.01a	-	-
North Wolo (23)	1.2±0.6b	-	0.172±0.01a	1.23±0.35b	0.34±0.14a	0.0400±0.01a	-	-
F value	2.20	1.06	29.07	4.10	3.85	5.22	0.47	0.42
P value	0.0371	0.4000	<.0001	0.0292	0.0457	<.0001	0.6345	0.7415
Growth stage								
Seedling	0.6±0.5c	0.0±0.1c	0.001±0.01c	0.31±0.32d	0.25±0.11c	0.014±0.01b	0.0±0.0b	-
Knee height	2.6±0.4b	0.6±0.3ab	0.001±0.02c	2.25±0.33b	0.45±0.11b	0.010±0.01b	0.0±0.0b	0.10±0.05a
Tasseling/ Flag leaf†	2.4±0.6b	0.9±0.3a	0.131±0.04a	1.78±0.51ac	0.56±0.18ac	0.023±0.02ab	0.51±0.31a	0.19±0.05a
Grain filling	3.3±0.4a	0.9±0.5a	0.082±0.01a	2.71±0.31a	0.61±0.10a	0.039±0.01a	0.01±0.53b	0.08±0.10ab
Harvest	0.9±0.4c	0.4±0.3b	0.081±0.01a	0.47±0.31cd	0.51±0.10ab	0.007±0.008b	0.44±0.28a	0.01±0.03b
F value	112.10	9.49	5.68	111.56	28.245	8.32	7.77	19.15
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
*Variety***								
Long maturing	4.4±0.2a	-	0.23±0.06b	4.2±0.19a	0.17±0.01a	0.05±0.004a	-	-
Medium maturing	1.5±0.3b	-	1.26±0.18a	1.43±0.38b	0.04±0.02b	0.02±0.005b	-	-
Early maturing	1.9±0.2b	-	1.30±0.12a	1.79±0.31b	0.09±0.02b	0.03±0.007b	-	-
F avlue	15.74	-	28.57	34.02	15.74	15.24	-	-
P value	<.0001	-	<.0001	<.0001	<.0.0001	<.0001	-	-

† Flag leaf for sorghum and tasseling for maize; - indicates no data or minor crop status; DE stands for the pest does not occur; ** 1st four administrative zones are in the cool-wet western Amhara (cool and wet climate), 2nd four in semi-arid eastern Amhara (semi-arid climate); *** long maturing stands for 8-9 month, medium for 6-7 month and early for 4-5-month varieties of sorghum only; Y stands for Year, Z for zone and D for district. Means followed by the same letter(s) within a column of a fixed effect are not significantly different according to Tukey-Kramer (P < 0.05).

flavipes was the predominant parasitoid species and it was only found in the *C. partellus*-dominated semi-arid eastern Amhara (North Shoa, Oromiya, South Wolo and North Wolo zones) (tab.2). Number of cocoon masses per plant and percent parasitism was highest in North Shoa and lowest in North Wolo zone. Larval parasitism was similar during both years though cocoon mass numbers were higher in 2003 than 2004. Larval parasitism increased with crop stage up to grain filling and then decreased at harvest. Long maturing sorghum varieties had significantly higher parasitism by *C. flavipes* and cocoon mass density than medium maturing ones (tab.2). In addition to *C. flavipes*, ten species of parasitoids and one hyperparasitoid, *Aphanogmus fijiensis* (Ferriere)

Table 2. Effects of year, location and crop growth stage on abundance of cocoon masses and earwigs per plant, on level of larval parasitism by C. flavipes and nematodes in the Amhara state, in 2003 and 2004.

	Sorghum				Maize	
	Cocoon masses/ plant	% Larval parasitism	Number of earwigs/ plant	Nematodes / plant	Nematodes / larva	Nematodes / plant
Random effects	% Variation explained					
Locality (district x zone x year)	21.2	16.5	14.1	7.9	2.2	3.1
Zone (year)	4.9	4.6	0.6	1.8	0.2	0.0
District	2.6	1.6	0.0	0.0	0.0	0.3
Residual	71.3	77.3	85.2	90.4	97.6	96.6
Fixed effects	Least square means (±SE)					
Year						
2003	0.7±0.06a	8.9±2.5	0.08±0.02a	0.01±0.005	0.005±0.003	0.004±0.04
2004	0.4±0.04b	11.3±2.0	0.02±0.00b	0.01±0.005	0.002±0.004	0.001±0.05
F value	9.48	0.58	64.57	0.22	0.62	0.12
P value	<.0001	0.4480	<.0001	0.6376	0.4313	0.7271
Administrative zone						
North Shoa	0.9±0.07a	23.4±5.5a	0.03±0.01a	0.003±0.010	0.001±0.004	-
Oromiya	0.5±0.05b	18.6±4.1ab	0.03±0.01a	0.010±0.004	0.002±0.003	-
South Wolo	0.5±0.06b	16.4±3.9bc	0.07±0.01a	0.010±0.004	0.003±0.003	-
North Wolo	0.3±0.06c	16.0±3.6c	0.03±0.01a	0.003±0.004	0.001±0.003	-
East Gojam	-	-	0.00±0.02b	0.010±0.010	0.010±0.004	0.01±0.11b
West Gojam	-	-	0.00±0.01b	0.010±0.030	0.010±0.025	0.05±0.07a
South Gondar	-	-	0.00±0.03b	0.010±0.010	0.010±0.010	0.02±0.14b
North Gondar	-	-	0.00±0.02b	0.010±0.010	0.0001±0.004	0.01±0.10b
F value	19.68	6.28	8.81	0.41	0.60	5.40
P value	<.0001	0.0003	<.0001	0.8926	0.7519	0.0341
Growth stage						
Seedling	0.2±1.1b	0.0±2.7c	0.00±0.01c	0.001±0.01b	0.0004±0.004b	-
Knee height	0.2±0.2c	1.3±2.1c	0.00±0.01c	0.001±0.01b	0.0005±0.005b	0.06±0.04
Tasseling/ Flag leaf †	0.5±0.3b	24.0±1.4a	0.01±0.01b	0.040±0.012a	0.0240±0.009a	0.00±0.04
Grain filling	1.3±0.13a	28.5±1.2a	0.01±0.01b	0.002±0.005b	0.0014±0.004b	0.00±0.09
Harvest	0.4±0.13b	14.8±1.4b	0.05±0.01a	0.000±0.010b	0.0000±0.004b	0.00±0.03
F value	25.24	66.74	9.14	17.38	9.82	2.17
P value	<.0001	<.0001	<.0001	<.0001	<.0001	0.0926
*Variety**						
Long maturing	0.8±0.07a	16.8±1.4a	0.03±0.02b	-	-	-
Medium maturing	0.1±0.14b	1.1±2.7b	0.01±0.04b	-	-	-
Early maturing	0.6±0.11a	15.5±2.2a	0.27±0.03a	-	-	-
F value	10.97	13.37	24.59	-	-	-
P value	<.0001	<.0001	<.0001	-	-	-

† Flag leaf for sorghum and tasseling for maize; - indicates no data or minor crop status; means followed by the same letter(s) within a column of a fixed effect are not significantly different according to Tukey-Kramer (P<0.05).

Table 3. Hymenopteran parasitoids of stem borers on cereal crops in different areas of the Amhara State, Ethiopia, in 2003 and 2004.

Family	Species	# Parasitoid	% Parasitism*	Host borer reared from	Behavior	Zone	Region
Braconidae	*Cotesia flavipes* Cameron	Thousands	29.00	*C. partellus larvae*	Gregarious	All zones	EA
Braconidae:	*Cotesia sesamiae*	2	0.7	*B. fusca* larvae	Gregarious	East Gojam	WA
Braconidae	*Euvipio rufa* Szepligeti	1	0.7	*B. fusca*	Solitary	North Gondar	WA
Braconidae	*Dolichogenidea*	1	0.2	*B. fusca* larvae	Gregarious**	South Gondar	WA
Braconidae	*Cotesia sesamiae*	1	0.7	*B. fusca* larvae	Gregarious	East Gojam	WA
Braconidae	*Dolichogenidea fuscivora* Walker	1	2.2	B. fusca	Gregarious	North Gondar	WA
Braconidae	*Dolichogenidea fuscivora* Walker	1	2.2	*B. fusca***	-	North Gondar	WA
Braconidae	*Dolichogenidea fuscivora* Walker	1	0.2	*B. fusca*	Gregarious**	South Gondar	WA
Ceraphronidae:	*Aphanogmus fijiensis* Ferriere	1	0.7	*C. partellus*	Gregarious	North Wolo	EA
Eulophidae	*Pediobius furvus* Gahan	1	2.3	*C. partellus* pupa	Gregarious	Oromiya	EA
Eulophidae	*Pediobius furvus* Gahan	1	1.0	*C. partellus* pupa	Gregarious	South Wolo	EA
Eulophidae	*Pediobius furvus* Gahan	1	0.4	*B. fusca* pupa	Gregarious	North Wolo	EA
Ichneumonidae		1		Unidentified pupa	Solitary	West Gojam	WA
Ichneumonidae		1		*B. fusca* pupa	Solitary	West Gojam	WA
Ichneumonidae		1		*B. fusca*	Solitary	West Gojam	WA
Ichneumonidae	*Dentichasmias busseolae* Heinrich	1	1.0	*C. partellus* pupa	Solitary	South Wolo	EA
Ichneumonidae	*Procerochasmias nigromaculatus* Cameron	2	1.3	*B. fusca*	Solitary	West Gojam, North Gondar	WA
Ichneumonidae	*Dentichasmias busseolae* Heinrich	1	0.2	*C. partellus* pupa	Solitary	Oromiya	EA

* percentage parasitism was calculated for individual survey sites, where the parasitoids were found, except for *Co. flavipes* which occurred widely;
** empty cocoon masses from which adult parasitoids have emerged were discovered; EA semi-arid eastern Amhara, WA the cool-wet western Amhara.

Table 4. Taylor's power law coefficients of borers at various growth stages of sorghum in the two regions of the Amhara state.

	Crop growth stage	Intercept (log a)	Slope (b)	r^2	P >F
Eastern Amhara	**Sorghum**				
	Ch. partellus				
	Seedling (belg)	0.78	1.22*a	0.93	<.0001
	Knee height	0.47	1.27*a	0.84	<.0001
	Grain filling	0.49	1.10a	0.66	<.0001
	Harvest	0.22	0.91a	0.76	<.0001
	B. fusca				
	Knee height	0.65	1.27*a	0.92	<.0001
	Grain filling	−0.06	0.97a	0.48	0.0182
	Harvest	−0.09	0.53*b	0.99	<.0001
	Pooled	0.28	1.64*a	0.79	<.0001
	S. calamistis				
	Seedling	0.40	1.06b	0.88	0.0060
	Knee height	0.35	1.10b	1.00	<.0001
	Grain filling	0.63	1.27*a	0.93	<.0001
	***C. flavipes* cocoon masses**				
	Knee height	0.69	1.27*a	0.87	<.0001
	Grain filling	0.33	1.17*a	0.91	<.0001
	Harvest	0.63	1.15b	0.65	0.0027
Western Amhara	**Maize**				
	B. fusca				
	Pooled	0.34	1.10	0.66	<.0001

* Slope different from 1, F-test, P<0.001; for each species separately slopes followed with the same letter(s) are not significantly different (P=0.05).

(Ceraphronidae) - belonging to five hymenopteran families were recorded (tab. 3). However, compared to *C. flavipes*, the rates of parasitism were exceedingly low (< 3%).

Number of earwigs varied significantly with administrative zone; it was higher during 2003 than 2004 and it increased with crop growth stage until

harvest. Residual or between plant variance contributed more than 70% of the overall variation followed by L x D x Z x Y (tab. 2).

In much of the cool-wet western Amhara, unidentified nematode species parasitized medium *B. fusca* larvae during the wet months between August and September in both years, which was lower in 2004

Table 5. Effect of year, location and crop growth stage on damage variables on sorghum and maize in the Amhata state, in 2003 and 2004.

	Sorghum			Maize			
	% Stem tunneling	% Internode damage	# Holes/ plant	% Stem tunneling	% Internode damage	# Holes/ plant	% Cob damage
Random effects	% variation explained						
Locality (year x zone x district)	32.9	45.5	37.0	16.5	23.0	25.5	11.5
Zone (year)	17.7	9.3	13.9	10.9	0.0	11.3	0.0
District	0.4	1.1	1.4	2.0	24.2	15.5	5.9
Residual	49.0	44.1	47.7	70.6	52.8	47.7	82.6
Fixed effects *Year*	Least square means (±SE)						
2003	13.2±2.4a	24.4±3.9a	6.5±1.2a	7.6±1.8a	24.5±5.1a	4.2±1.5	0.3±1.8a
2004	7.9±2.1b	17.9±3.2b	3.2±1.2b	5.1±1.9a	17.1±4.3b	2.4±1.6	1.4±1.5a
F value	7.20	4.93	10.89	3.73	30.66	2.65	0.35
P value	0.0081	0.0280	0.0012	0.0621	<.0001	0.1129	0.5596
Administrative zone							
East Gojam	8.3±0.5d	8.5±0.9b	0.5±0.2bc	5.4±2.0	20.7±5.5	5.3±1.7	0.3±2.2
West Gojam	0.0±0.0e	0.0±0.0c	0.0±0.0c	6.3±1.5	20.4±4.2	5.2±1.2	1.8±1.5
South Gondar	12.7±1.3cd	9.5±1.3b	0.1±0.2bc	9.3±3.8	21.4±9.2	5.5±3.2	0.6±3.1
North Gondar	21.5±0.7ab	22.8±0.8a	2.3±0.2bc	-	-	-	-
North Shoa	26.2±1.2a	40.2±1.3a	11.6±0.6a	-	-	-	-
Oromiya	18.7±0.7ac	36.0±0.9a	10.6±0.4a	-	-	-	-
South Wolo	16.9±0.7ad	31.1±0.9a	9.0±0.4a	-	-	-	-
North Wolo	10.0±0.5cd	31.2±0.8a	7.4±0.2b	-	-	-	-
F value	74.59	109.82	121.09	2.03	0.01	0.76	0.19
P value	<.0001	<.0001	<.0001	0.1297	0.9940	0.5602	0.8332
Growth stage							
Seedling	0.0±0.0d	0.0±0.0c	0.0±0.0d	-	-	-	
Knee height	3.4±0.4c	17.4±1.2b	2.4±0.3c	7.3±0.8a	-	1.5±0.3b	-
Flag leaf/ Tasseling	17.8±0.9b	37.0±4.2a	7.7±0.2b	6.2±0.6ab	32.2±4.7a	4.2±0.4a	-
Grain filling	22.3±0.7a	32.1±1.0a	10.3±0.4a	5.8±1.9b	26.1±4.1a	4.4±1.9a	-
Harvest	19.6±0.5b	24.7±0.5b	7.3±0.2b	0.2±0.3c	9.4±4.1b	0.0±0.2c	-
F value	244.69	531.70	193.26	35.65	107.44	38.78	-
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	-
Variety*							
Long maturing	38.6±1.2a	50.6±1.1a	20.6±0.7a	-	-	-	-
Medium maturing	21.5±2.3b	30.4±2.2b	12.1±1.4b	-	-	-	-
Early maturing	18.4±1.8b	33.1±1.8b	9.7±1.14b	-	-	-	-
F value	54.0	55.42	38.33	-	-	-	-
P value	<.0001	<.0001	<.0001	-	-	-	-

† Flag leaf for sorghum and tasseling for maize; - no data available or minor crop status in the area; maize is uncommon in North Gondar and sampling was not enough to present data; * differences in varietal duration to maturity were more substantial in sorghum than maize means within a column of a fixed effect followed by the same letter(s) are not significantly different from each other according to Tukey-Kramer (P < 0.05).

than 2003. Nematodes were also discovered from a few borers in semi-arid eastern Amhara in 2004. Sampling error contributed more than 90% of the variation in nematode population (tab. 2).

Between plant distribution of borers and their natural enemies in maize and sorghum

In semi-arid eastern Amhara, for *C. partellus* on sorghum, the regressions between log (mean) and log (variance) yielded significantly higher coefficients of determination (r^2) at seedling, knee height, grain filling and harvest stages. The slopes (*b*) were greater than 1 at seedling, knee height, grain filling stages, indicating an aggregated distribution of *C. partellus*, while at harvest, a random distribution was observed (tab.4). All slopes were not significantly different from each other. When pooled across crop growth stages, an aggregated distribution (b = 1.32, r^2 = 0.87, *P* = 0.001) was obtained. Similarly, an aggregated distribution was found for *S. calamistis* at the seedling knee height and grain filling stages. *C. flavipes* cocoon masses showed an aggregated distribution across all growth stages. *B. fusca* had variable distribution from random to regular

to aggregated at different crop stages while the pooled data gave highly aggregated distribution. In the cool-wet region, on maize, the pooled data of *B. fusca* had aggregated distribution (tab.4).

Plant damage variables

In general, plant damage variables followed the same trend as borer numbers. On sorghum, percentage stem tunneling, internode damaged and number of holes per plant were higher during 2003 than 2004, higher in eastern than the cool-wet western Amhara, and tended to increase with crop growth stage (tab.5). There was a general trend of decrease in damage levels from North Shoa northwards to North Wolo. Long maturing varieties had significantly higher damage levels. On sorghum, the sums of the contribution of the random effects of Locality x D x Z x Y and Z x Y was more than the contribution of variation by the residual in borer damage (tab.5). On maize, the residual contributed 48–83% of the variation in borer damage symptoms.

On maize, percent internodes damaged varied significantly with year, while the number of holes/plant

Table 6. Effect of year and location on sorghum and maize yield and yield components in the Amhara state, in 2003 and 2004.

	Sorghum		Maize	
	Head weight (g/ plant)	Grain yield (kg/ ha)	Cob weight (g/ plant)	Grain weight (kg/ ha)
Random effects	**% Variation explained**			
Locality (district x zone x year)	28.3	100.0	51.7	41.0
District	12.1	0.0	0.0	0
Residual	59.6	0.0	48.3	59.0
Fixed effects	**Least square means (±SE)**			
Year				
2003	63.8±1.4a	1833.1±181.1a	206.3±10.1a	3837.1±865.9a
2004	57.9±2.0b	1891.4±228.2a	167.7±9.1b	3724.6±834.3a
F value	152.10	0.04	9.57	0.01
P value	<.0001	0.8472	0.0021	0.9926
Administrative zones				
East Gojam	70.2±2.9b	1932.9±340.9ab	155.9±11.8b	3438.5±234.4
West Gojam	-	1271.4±191.5b	232.0±7.6a	5913.2±367.1
South Gondar	11.0±3.9c	1321.0±334.0b	173.1±17.1b	2140.8±402.2
North Gondar	17.7±1.7c	1261.4±404.2b	*	*
North Shoa	68.2±3.9b	1721.6±475.5ab	*	*
Oromiya	67.7±2.7b	2281.1±294.6ab	*	*
South Wolo	70.8±3.0b	1668.8±336.9b	*	*
North Wolo	120.2±3.8a	2867.3±377.1a	*	*
F value	6.61	2.54	16.05	4.99
P value	<.0001	0.0298	<.0001	0.0561

* crop not grown or minor status means within a column of a fixed effect followed by the same letter(s) are not significantly different from each other according to Tukey-Kramer (P < 0.05).

varied significantly with crop growth stages (tab.5) In most cases, significantly higher damage levels were recorded at around tasseling and grain filling than other growth stages (tab.5). Cob damage was generally low and non-significant; residual or between-plant variance contributed most to the overall variability followed by district (tab.5).

Yield and yield components

Head weight of sorghum and cob weight of maize were significantly higher in 2003 than 2004 (tab. 6). Moreover, sorghum grain yields varied significantly with administrative zone. More than twofold sorghum yield was obtained in North Wolo than in North or South Gondar (tab. 6). The highest mean maize grain yield was obtained in West Gojam zone. On sorghum, random effects and residual contributed about 40% and 60% to the variation in head weight, respectively (tab. 6). On maize, both the random effects and the residual contributed equally in both cob and grain weight.

Relationships between borers, parasitism, borer damage, plant growth variables, altitude and yield

Abundance of B. fusca was significantly and positively correlated with altitude (r = 0.27, P < 0.05) while C. partellus, borer damage levels, and larval parasitism by C. flavipes were significantly and negatively related to altitude (r = –0.18 to –0.46, P< 0.05). All plant growth parameters, borer numbers, and parasitism were significantly and positively correlated with crop growth stage (r = 0.14 to 0.70, P < 0.05).

Table 7. Effect of borer damage variables and plant growth parameters on head weight of maize and sorghum in the Amhara state, Ethiopia, in 2003 and 2004.

	b	F value	P value
Sorghum			
Y head weight (g/ plant)			
X_1 plant height (m)	28.19	73.13	<.0001
X_2 stem diameter (cm)	50.86	190.72	<.0001
X_3 internodes/ plant	–3.96	29.38	<.0001
X_4 % peduncle damage	–0.40	19.54	0.0089
X_5 % tunneling	–0.20	0.51	0.0451
X_6 # holes/ plant	–0.44	6.86	0.0358
X_7 % larval parasitism	0.13	4.42	0.0358
Intercept = -5.11, r^2 = 0.29, N = 6680			
Maize			
Y cob weight (g/ plant)			
X_1 plant height (m)	173.0	107.19	<.0001
X_2 stem diameter (cm)	150.53	88.21	<.0001
Intercept = –421.2, r^2 = 0.69, N = 207			

* Stepwise multiple regressions; *b* values are partial regression coefficients.

Furthermore, plant growth variables, damage and borers were all significantly correlated with each other. Yield per plant was significantly negatively correlated with percent tunneling and B. fusca population (r = -0.05, P < 0.05).

A stepwise regression analysis indicated that sorghum head weight was positively related to plant height, stem diameter and parasitism, and negatively to percent peduncle damage, number of holes per plant, and % stem tunneling (tab.7). On maize, cob weight was positively related to plant height and stem diameter only, while plant damage and insect variables had no effect.

Discussion

In the present study, three lepidopteran, i.e., *Chilo partellus, Busseola fusca and Sesamia calamistis*, and one coleopteran, *Rhynchaenus niger*, borer species were found in the Amhara state of Ethiopia. In addition to these borer species, Emana *et al.* (2001) reported others including the lepidopteran *Sesamia nonagrioides botanephaga*, and the coleopteran *Pissodius dubius* in Ethiopia. The coleopteran borers are recent records (Emana 2002) and they were observed attacking sorghum. Their economic importance is virtually unknown. They are known as flee weevils and feed by boring wild trees (Anonymous 2000). The recent and present findings suggested that these beetles are shifting to cultivated crops. B. fusca dominated the cool-wet western and *Ch. partellus* the warm semi-arid eastern Amhara, indicating differences in their environmental requirements (Tessema 1982; Assefa 1985). *S. calamistis* was commonly observed at the vegetative stages of maize and when moisture was abundant suggesting its sporadic status (Assefa 1991). In Cameroon, *S. calamistis* was reported attacking mostly pre-tasseling stages (Ndemah & Schulthess 2002) and maize ears in Uganda (Kalule *et al.* 1997). In the humid zones of West Africa, it is the key noctuid pest of maize (Schulthess *et al.* 1997).

Correlation analyses showed that the abundance of B. fusca increased with altitude. In East Gojam (i.e., cool-wet ecozone), B. fusca populations were found as high as 2600 m corroborating earlier studies in southern, central, eastern and western Ethiopia (Assefa 1985; Emana *et al.* 2001). C. partellus, on the other hand, was found to be negatively correlated with altitude. In semi-arid eastern Amhara, C. partellus was found up to 1900 m elevation, corroborating findings by Emana *et al.* (2001) on the distribution of stem borers in Ethiopia. Studies elsewhere in East and southern Africa also indicate that C. partellus and B. fusca are low to medium and high elevation borer

species, respectively (Ingram 1958; Seshu Reddy 1983; Assefa 1985; Overholt *et al.* 1997; Haile & Hofsvang 2001). Based on the geographic information systems, in the Amhara state, *C. partellus* is predicted to occur east of Ethiopia's eastern escarpment (western bank of the Rift Valley), i.e., areas below 1800 m elevation, and in the western districts of the Amhara state bordering Sudan, i.e., < 800 m (Emana *et al.* 2002; Muchugu, ICIPE, Nairobi, *pers. comm.*). Emana *et al.* (2002) showed that rainfall and temperature play significant role in *C. partellus* distribution. In eastern Amhara, the dry winds from the east must have modifying effect in favour of *C. partellus* on altitudes as high as 2000 m (Muchugu, ICIPE, Nairobi, Kenya, *pers. comm.*). In South Africa, *Ch. partellus* was increasingly invading the cooler areas, displacing *B. fusca* (Kfir 1997). Similar displacement might have happened in eastern Amhara. *C. partellus* has been in eastern Amhara since at least the 1970s and has never climbed up the nearby plateau, although it managed to invade the nearby high altitudes as high as 1900 m. So far, *C. partellus* was not found in the mid and high altitudes of western Amhara.

The extensive cold plateau, i.e., the high elevations west of the Rift Valley, traditionally known as Western Highlands, stretch from northeast to southwest of the state and create a long, wide buffer zone between semi-arid eastern and cool-wet western Amhara, probably preventing *C. partellus* from invading west. Geographical barriers (mountain ranges and forests) play significant role in limiting the distribution and economic importance of borers and their enemies among African regions and ecozones (Schulthess *et al.* 1997). Similarly, the major pest in Cameroon is *B. fusca* (Ndemah *et al.* 2001a) while in the rest of West Africa, which is separated from Cameroon by mountain ranges and swamps, the key pests are *Eldana saccharina* (Walker) and *S. calamistis* (Schulthess *et al.* 1997). Also, because of dense humid forests and lack of east-west thorough roads, *C. partellus* has not yet spread to most of Central and West Africa.

In West Gojam zone, *S. calamistis* replaced *B. fusca* in maize fields near Lake Tana, especially during the flag leaf stage. The Lake Tana area of West Gojam zone is warmer than other nearby zones, where *B. fusca* dominates. Shanower *et al.* (1993) reported that maize is the best host for *S. calamistis* development and survival.

Levels of infestation varied significantly with year, location, growth stage, crop type and variety. Infestation was higher in 2003 than 2004. The first effective rainfall and the total rainfall in the planting month of June in some representative locations in the cool-wet western Amhara was 26-33% higher in 2003 than at the same time in 2004. This higher rainfall could have caused an early and increased emergence of larvae from diapausing larvae in 2003 than 2004. Borer populations are strongly influenced by amount and distribution of rainfall (Cardwell *et al.* 1997; Ndemah *et al.* 2000; Ndemah & Schulthess 2002). On one hand, young larvae feeding in the whorl might drown, on the other hand, sufficient soil water might increase the vigour of the plant and thereby survival of young larvae (Sétamou *et al.* 1995).

Crop residues are stacked for animal feed and construction purposes guaranteeing the steady maintenance of stem borers in the two regions. They harbor diapausing larvae during the off-season and, thus, are major source of infestation (Van den Berg *et al.* 1998; Harris & Nwanze 1992; Assefa 1988a; 1988b; Adesiyun & Ajayi 1980). Stem borers were minor in South Gondar, East and West Gojam zones. Farmers in these areas plough their fields immediately after harvest. Furthermore, nematodes and the high rainfall may also contribute to reducing borer infestations.

Long maturing varieties had higher infestations due to long time of exposure to borers allowing for more than one generation on the same plant. This corroborates results by Van den Berg *et al.* (1990) and Tanzubil *et al.* (2002).

C. flavipes was the most abundant parasitoid species in semi-arid eastern Amhara with an average larval parasitism of up to 30%. Emana *et al.* (2001) reported maximum rates of around 7.5% between 1999 and 2000, which indicates that parasitism is on the increase. Back in 1998, when Mulugeta (2001) surveyed several cereal growing areas of Ethiopia, *C. flavipes* was not yet present. Consequently, *C. flavipes* must have invaded Ethiopia in 1999, probably from Somalia, where it was released in 1997 (Emana *et al.* 2001; Emana *et al.* 2003). Its advance westwards to the cool-wet western Amhara has been hindered by the physical barrier, the Western Highlands (plateau), that also prevented *C. partellus* from moving west. Though *C. flavipes* is very abundant in semi-arid eastern Amhara, borer populations and their effects are still high. Zhou *et al.* (2001) reported that in Coastal Kenya it took five years before *C. flavipes* had a significant effect on *C. partellus* infestations. Thereafter, densities decreased by around 70%. Larval parasitism tended to be higher on long maturing varieties. Several indigenous larval and pupal parasitoids were also observed but parasitism was below 2%. Earlier reports showed that parasitism of *C. partellus* and *B. fusca* by *C. sesamiae* outside of the Amhara State varied considerably, i.e., 0 to 1.2% (Emana *et al.* 2001); 25% (Assefa 1985) and >25%

(Mulugeta 2001). By contrast, Kfir (1995) reported 90% parasitism of *B. fusca* by *Co. sesamiae* and up to 100% parasitism of pupae by *Dentichasmias busseolae* and *Pediobius furvus* of up to 100% in South Africa.

Nematodes were observed during the wet months in the cool-wet western Amhara. Wet habitats are essential for nematode survival (Kaya & Gaugler 1993; Poinar Jr & Polaszek 1998). Nematodes might have contributed to low *B. fusca* infestations in the cool-wet western Amhara. Similarly, Emana *et al.* (2001) reported higher nematode densities (i.e., *Steinernema intermedia*) on *B. fusca*. In Africa, 16 species of nematodes are recorded to attack cereal stem borers (Poinar Jr & Polaszek 1998). Both crop damage levels and grain yields were higher in eastern than the cool-wet western Amhara; the two regions vary greatly in, among others, crops and varieties, soil fertility and drainage, climate and borer species. Sorghum yields were negatively affected by tunneling, holes bored and peduncle damage across region. Stem tunnelling (Bosque-Perez & Mareck 1991; Van den Berg *et al.* 1991; Kalule *et al.* 1994; Setamou *et al.* 1995; Kalule *et al.* 1997; Songa *et al.* 2001; Ndemah & Schulthess 2002; Chabi-Olaye *et al.* 2005a) and bored internodes (Macfarlane 1990) were considered as good indicators of the degree of plant damage and, thus, yield loss. Yields were also negatively related to *B. fusca* larval density corroborating previous reports (Ndemah & Schulthess 2002; Chabi-Olaye *et al.* 2005a). In contrast, Ndemah *et al.* (2001a) reported no relation between cob yield and borers because borers migrate, reach adulthood, or get killed by natural enemies.

When pooled across plant growth stages and, thus, age of borers, *C. partellus*, *B. fusca* and *S. calamistis* larvae and pupae had an aggregated distribution. Similar findings were reported from *C. partellus* (Overholt *et al.* 1994b), on *B. fusca* (Chabi-Olaye *et al.* 2005b) and on *S. calamistis* (Schulthess *et al.* 1991). The distributions become progressively less aggregated as insects aged (Overholt *et al.* 1994b; Ndemah *et al.* 2001b). Stem borer eggs are laid in batches, thus eggs and young larvae are pseudo-aggregated. The decrease in aggregation with age of larvae indicates dispersal of larvae. In fact, second instar larvae migrate from the oviposition site inside the leaf sheaths to the whorl where they feed on the leaves or balloon off to other plants (Berger 1989, 1993; Päts & Ekbom 1992). Older larvae migrate if plant vigour is affected by stem tunneling.

Conclusion

The objective of this study was to determine the distribution and importance of stem borers and their natural enemies in the Amhara State of Ethiopia. The results show that *C. partellus* and *B. fusca* were the major borers, while *Co. flavipes* and nematodes were the major natural enemies in the semi-arid eastern and cool-wet western ecozones of the Amhara State, respectively. Knowledge of the composition and status of borers and natural enemies provides the foundation for any pest management. This gives a great opportunity to tackle the two major pests by *Co. flavipes* and nematodes in the two regions (ecozones) of the State. To that end, impact assessment of *Co. flavipes* and its supplementary release in isolated areas like the cool-wet western Amhara, and population dynamics of both major borers and their natural enemies in representative locations of two regions are suggested.

References

Adesiyun A.A., Ajayi O. 1980. Control of the stem borer, *Busseola fusca*, by partial burning of stalks. *Tropical Pest Management* 26: 113-117.

Anonymous 2000. Family Curculionidae (weevils). *http://cedarcreek.umn.edu/insects/024113n.html.*

Assefa G. 1985. Survey of lepidopterous stem borers attacking maize and sorghum in Ethiopia. *Ethiopian Journal of Agricultural Sciences* 7: 15-26.

Assefa G. 1988a. Development of maize stalk borer, *Busseola fusca* (Fuller) in wild host plants in Ethiopia. *Journal of Applied Entomology* 106: 390-395.

Assefa G. 1988b. Survival of maize stalk borer, *Busseola fusca* (Fuller) in crop residues in Ethiopia. *Crop Protection* 7: 181-185.

Assefa G. 1989. Phenology and fecundity of maize stalk borer, *Busseola fusca* (Fuller) in Awassa, southern Ethiopia. *Insect Science and its Application* 10: 131-137.

Assefa G. 1991. Maize entomology in Ethiopia, p. 27-29 *in*: Tsedeke A., Teferi G., Abdurrahman A. (eds.), *Proceedings of the 11th annual meeting of the Committee of Ethiopian Entomologists (CEE)*, 21-22 February 1991, Addis Ababa, Ethiopia. CEE: Addis Ababa.

Berger A. 1989. Ballooning activity of *Chilo partellus* larvae in relation to size of mother, egg batches, eggs and larvae and ages of mother. *Entomologia Experimentalis et Applicata* 50: 125-132.

Berger A. 1993. *Larval migration in the stem borer* Chilo partellus *(Lepidoptera: Pyralidae)*. Thesis, Swedish University of Agricultural Science, Sweden, 78 p.

BOA (Bureau of Agriculture) 1999. *Agricultural research master plan of the Amhara State, crop protection*. Vol II, part 9. Technical report. BOA, Bahir Dar, Amhara State, Ethiopia, 59 p.

Bosque-Perez N.A., Mareck J.H. 1991. Effect of stem borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) on the yield of maize. *Bulletin of Entomological Research* 81: 243-247.

CACC (Central Agricultural Census Commission) 2003. *Ethiopian agricultural sample enumeration, 2001/02 (1994 E.C.), results at country level. Statistical report on socio-economic characteristics of the population in agricultural households, land use, and area and production of crops.* Part I. CSA, Addis Ababa, Ethiopia.

Cardwell K.F., Schulthess F., Ndemah R., Ngoko Z. 1997. A systems approach to assess crop health and maize yield losses due to pests and diseases in Cameroon. *Agriculture, Ecosystems and Environment* 65: 33-47.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005a. Relationships of intercropping maize, stem borer damage to maize yield and land-use efficiency in the humid forest of Cameron. *Bulletin of Entomological Research* 95: 417-427.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005b. Abundance, dispersion and parasitism of the stem borer *Busseola fusca* (Lepidoptera: Noctuidae) in maize in the humid forest zone of southern Cameroon. *Bulletin of Entomological Research* 95: 169-177.

CSA (Central Statistical Authority) 2000. *Agricultural sample survey 1999/2000: report on area and production for major crops (private peasant holdings, Meher season).* Statistical bulletin No. 171. CSA: Addis Ababa, Ethiopia, 73 p.

Emana G. 1998. *Insecticide screening against maize stalk borer,* Busseola fusca *(Fuller).* Proceedings of the 6th annual conference of the Crop Protection Society of Ethiopia (CPSE), 3-5 June 1998, Addis Ababa. CPSE: Addis Ababa.

Emana G., Overholt W.A., Kairu E.W. 2001. Distribution and species composition of stem borers and their natural enemies in maize and sorghum in Ethiopia. *Insect Science and its Application* 21: 353-359.

Emana G. 2002. Ecological analysis of stem borers and their natural enemies under maize and sorghum based agroecosystems in Ethiopia. PhD Dissertation, Kenyatta University, Nairobi, Kenya, 261 p.

Emana G., Overholt W.A., Kairu E., MacOpiyo L., Zhou G. 2002. Predicting the distribution of *Chilo partellus* (Swinhoe) and parasitoid *Cotesia flavipes* Cameron in Ethiopia using correlation, stepwise regression and geographic information systems. *Insect Science and its Application* 22: 123-129.

Emana G., Overholt W.A., Kairu E.W., Omwega C. 2003. Evidence of the establishment of *Cotesia flavipes* (Hymenoptera: Braconidae), a parasitoid of cereal stem borers, and its host range expansion in Ethiopia. *Bulletin of Entomological Research* 93: 125-129.

FAO (Food and Agriculture Organization of the United Nations). 2003. *Production Yearbook for 2002.* Rome, Italy.

Ferdu A., Demissew K., Birhane A. 2001. *Major insect pests of maize and their management: A review.* Proceedings of the second national maize workshop of Ethiopia, CIMMYT/EARO, Addis Ababa, Ethiopia, p. 89-96.

Gashawbeza A., Melaku W. 1996. Loss caused by stalk borers, *Chilo partellus* (Swinhoe) and *Busseola fusca* (Fuller) on sorghum in central Ethiopia, p. 48-53 *in:* Eshetu B., Abdurrahman A., Aynekulu Y. (eds.), *Proceedings of the third annual conference of the Crop Protection Society of Ethiopia (CPSE),* 18-19 May 1995, Addis Ababa, Ethiopia. CPSE: Addis Ababa.

Haile A., Hofsvang T. 2001. Survey of lepidopterous stem borer pests of sorghum, maize and pearl millet in Eritrea. *Crop Protection* 20: 151-157.

Harris K.M., Nwanze K.E. 1992. Busseola fusca *(Fuller), the African maize stalk borer: a handbook of information.* Information Bulletin no. 33. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics, and Wallingford, UK: Cab International, 92 p.

Ingram W.R. 1958. The lepidopterous stalk borers associated with Gramineae in Uganda. *Bulletin of Entomological Research* 49: 367-383.

Kalule T., Ogenga-Latigo M.W., Okoth V.A.O. 1994. Relationship between successive dates of sowing maize and stem borer damage in central Uganda. *Proceedings of African Crop Science Conference* 1: 338-340.

Kalule T., Ogenga-Latigo M.W., Okoth V.A.O. 1997. Seasonal fluctuations and damage of lepidopteran stem borers of maize in a major agroecozone of Uganda. *African Crop Science Journal* 5: 385-393.

Kaya H.K., Gaugler R. 1993. Entomopathogenic nematodes. *Annual Review of Entomology* 38: 181-206.

Kfir R. 1995. Parasitoids of the African stem borer, *Busseola fusca* (Lepidoptera: Noctuidae), in South Africa. *Bulletin of Entomological Research* 85: 369-377.

Kfir, R. 1997. Competitive displacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America* 90: 619-624.

Macfarlane J.H. 1990. Damage assessment and yield loss in sorghum due to the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in northern Nigeria. *Tropical Pest Management* 36: 131-137.

Mulugeta N. 2001. Survey of parasitoids on lepidopterous stem borers attacking maize and sorghum in some localities of Ethiopia. *Pest Management Journal of Ethiopia* 5: 69-75.

Ndemah R., Schulthess F., Poehling H.M., Borgemeister C. 2000. Species composition and seasonal dynamics of Lepidopterous stem borers on maize and elephant grass, *Pennisetum purpureum* (Moench) (Poaceae), at two forest margin sites in Cameroon. *African Entomology* 8: 265-272.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Cardwell K.F. 2001a. Distribution, relative importance and effect of lepidopterous borers on maize yields in the forest zone and mid-altitude of Cameroon. *Journal of Economic Entomology* 94: 1434-1444.

Ndemah R., Schulthess F., Poehling M., Borgemeister C. 2001b. Spatial dynamics of lepidopterous pests on *Zea mays* (L.) and *Pennisetum purpureum* (Moench) in the forest zone of Cameroon and their implication for sampling schemes. *Journal of Applied Entomology* 125: 507-514.

Ndemah R., Schulthess F. 2002. Yield of maize in relation to natural field infestations and damage by lepidopteran borers in the forest and forest/savannah zones of Cameroon. *Insect Science and its Application* 22: 183-193.

Overholt W.A., Ngi-Song A.J., Kimani S.K., Mbapila J., Lammers P., Kioko E. 1994a. Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), in Africa. *Biocontrol News and Information* 15: 19-24.

Overholt W.A., Ogedah K., Lammers P.M. 1994b. Distribution and sampling of *Chilo partellus* (Lepidoptera: Pyralidae) in maize and sorghum on the Kenya coast. *Bulletin of Entomological Research* 84: 367-378.

Overholt W.A., Ngi-Song A.J., Omwega C.O., Kimani-Njogu, S.W., Mbapila J., Sallam M.N., Ofomata V. 1997. A review of the introduction and establishment of *Cotesia flavipes* (Cameron) in East Africa for biological control of cereal stem borers. *Insect Science and Its Application* 17: 79-88.

Päts P., Ekbom B. 1992. Infestation and dispersal of early instars of *Chilo partellus* (Lepidoptera: Pyralidae) at different densities. *Environmental Entomology* 21: 1110-1113.

PEDB (Planning and Economic Development Bureau) 1999. *Atlas of the Amhara National Region.* PEDB: Bahir Dar, Ethiopia, 119 p.

Poinar Jr. G.O., Polaszek A. 1998. Nematoda, fungi, protozoa, bacteria and viruses, p. 283-293 *in:* Polaszek A. (ed.), *African cereal stem borers: Economic importance, taxonomy, natural enemies and control.* CAB International, Wallingford, UK.

SAS Institute 1999-2000. *SAS/STAT user's guide. Version 8.01.* SAS Institute Inc., Cary, North Carolina, USA.

Schulthess F., Bosque-Perez N.A., Gounou S. 1991. Sampling lepidopterous pests on maize in West Africa. *Bulletin of Entomological Research* **81**: 297-301.

Schulthess F., Bosque-Perez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Goergen G. 1997. Exchange of natural enemies of lepidopteran cereal stem borers between African regions. *Insect Science and its Application* **17**: 97-108.

Seshu Reddy K.V. 1983. Studies on the stem borer complex of sorghum in Kenya. *Insect Science and its Application* **4**: 3-10.

Sétamou M., Schulthess F., Bosque Perez N.A., Thomas-Odjo A. 1995. Effect of stem and cob borers on maize subjected to different treatments. *Entomologia Experimentalis et Applicata* **77**: 205-210.

Shanower T., Schulthess F., Bosque-Perez N.A. 1993. The effect of larval diet on growth and development of *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and Eldana saccharina (Lepidoptera: Pyralidae). *Insect Science and its Application* **14**: 681-685.

Shegaw T., Libawit H.M., Bizuayehu A. 1999. *Survey of maize stalk borers and their bio-agents in south-western part of the Amhara National Regional State, Ethiopia.* Bahir Dar Plant Health Clinic, Bahir Dar, Ethiopia, 67 p.

Songa J.M., Guofa Z., Overholt W.A. 2001. Relationships of stem borer damage and plant physical condition to maize yield in a semi-arid zone of eastern Kenya. *Insect Science and its Application* **21**: 243-249.

Tadesse G. 1989. Research approach and monitoring pest management practices in Ethiopia, p. 108-119 *in: Proceedings of the 20ᵗʰ national crop improvement conference, 20-30 March 1987, Addis Ababa, Ethiopia.*

Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia.

Tanzubil P.B., Mensah G.W.K., McCaffery A.R. 2002. Effect of millet variety and date of sowing on diapause development in *Coniesta ignefusalis* (Hampson) in northern Ghana. *Crop Protection* **21**: 793-798.

Taylor L.R. 1961. Aggregation, variance and mean. *Nature* **189**: 732-735.

Tessema M. 1982. Insect pests of sorghum in Ethiopia, p. 54-64 *in: Proceedings of the regional workshop on sorghum improvement in eastern Africa, 17-21 October 1982.* Ethiopian Sorghum Improvement Project (ESIP), Debre Zeit, Ethiopia.

Van den Berg J., Van Rensburg J.B.J., Pringle K.L. 1990. Damage caused by *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) to various cultivars of grain sorghum, *Sorghum bicolor* (L.) Moench. *South African Journal of Plant Soil* **7**: 192-196.

Van den Berg J., Van Rensburg J.B.J., Pringle K.L. 1991. Comparative injuriousness of *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Pyralidae) on grain sorghum. *Bulletin of Entomological Research* **82**: 137-142.

Van den Berg J., Nur A.F., Polaszek A. 1998. Cultural control, p. 333-347 *in:* Polaszek A. (ed.), *African cereal stem borers: economic importance, taxonomy, natural enemies and control.* Wallingford, UK: CABI.

Zhou G., Baumgartner J., Overholt W.A. 2001. Impact assessment of an exotic parasitoid on stem borer (Lepidoptera) population dynamics in Kenya. *Ecological Application* **11**: 1554-1562.

Sexual dimorphism of antennal, tarsal and ovipositor chemosensilla in the African stemborer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae)

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Abstract. The number and distribution of chemosensilla located on different organs of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) males and females are described based on observations using scanning electron microscopy, selective staining with silver nitrate, and gustatory electrophysiological recording. The antennae and the fifth tarsomere of the prothoracic legs of both sexes bear chemosensilla: uniporous chaetica and multiporous trichoidea sensilla. However, there is a sexual dimorphism in the number and size of sensilla on these organs. The distal part of the ovipositor has uniporous gustatory chemosensilla of the chaetica type. The involvement of these sensilla in oviposition site selection by *B. fusca* is discussed.

Résumé. Dimorphisme sexuel des sensilles chémoréceptrices des antennes, des tarses et de l'ovipositeur du foreur de graminées africain, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). Le nombre et la distribution des sensilles chémoréceptrices présentes sur les différents organes sensoriels des mâles et femelles de *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) ont été étudiés sur la base d'observations en microscopie électronique à balayage, par leur réponse à une coloration sélective au nitrate d'argent et à l'électrophysiologie de contact. Les antennes et le cinquième article des tarses des pattes antérieures des deux sexes possèdent des sensilles chémoréceptrices identifiées respectivement comme de type chétiforme uni-poreux et comme de type trichoïde multi-poreux. Un dimorphisme sexuel est observé par rapport au nombre et à la taille de ces sensilles. La partie distale de l'ovipositeur possède également des sensilles chémoréceptrices gustatives chétiformes. Le rôle de ces structures chémoréceptrices dans la sélection du site de ponte chez *B. fusca* est discuté.

Keywords: Stem borer, host-plant selection, scanning electron microscopy, gustatory electrophysiological recording, Africa.

In East and Southern Africa, *Busseola fusca* (Fuller 1901) (Lepidoptera: Noctuidae) is the most important insect pest of sorghum and maize in the cooler ecozones such as the mid-altitude and highlands (Kfir *et al.* 2002). Various control strategies based on host plant resistance and cultural control have been tried, some with partial or local success, but none have provided a complete solution (Kfir *et al.* 2002). Recently, habitat-management strategies involving the use of "push-pull" or stimulo-deterrent diversionary tactics have been developed (Khan *et al.* 2000). Thereby, the stemborers are attracted and retained on

trap plants (pull) planted as border rows, repellent intercrops (push) prevent them from infesting the crop. The effective use of such strategies requires a good understanding of the host selection and acceptance processes by the insect pest.

Given the importance of the host plant selection process for the survival of the offspring, females of *B. fusca* very likely have special organs for detection of a wide range of cues including visual, tactile, and olfactory and gustatory chemostimuli. Preliminary studies indicated that females are nocturnal and that some of these cues may play a greater role in host plant location and acceptance than others (Calatayud *et al.* unpublished). The role of plant volatiles as long-range chemical cues is not clearly understood in *B. fusca*, however, these chemicals may facilitate host finding by stimulating take off and flight activity in mated females. After landing on host plant, tactile and

gustatory stimuli are likely to play a major role for the acceptance of the plant for oviposition. Physical and chemical characteristics of the oviposition site are involved in host plant acceptance for oviposition (Calatayud *et al.* unpublished).

The present study documents the distribution of chemosensory structures that may be involved in the process of oviposition site selection by *B. fusca* females and provides a basis for an interpretation on their role in oviposition choice. Sensilla located on the antennae and the fifth tarsomere of the prothoracic legs of both male and female *B. fusca* were compared. For females, sensilla on the ovipositor were also studied. Their physiological function was delineated based on the criteria proposed by Altner (1977) and Zacharuk (1985) with regard to morphological characteristics observed by scanning electron microscopy, penetration of dyes applied externally (Slifer 1960; Nayak & Singh 1983) and by electrophysiological tip recording (Marion-Poll *et al.* 1992).

Material and methods

Insects

Busseola fusca males and females were provided by the Animal Rearing and Containment Unit of the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). They were reared on a meridic diet (Onyango & Ochieng'-Odero 1994). To regenerate the colony, new insects collected in the field were added three times a year.

The male and female pupae were maintained separately until emergence in a plastic box (21 cm long, 15 cm wide, and 8 cm high). A piece of wet pad maintained the moisture at > 80 % r.h. in the box. The insects were maintained in a controlled room at 26.1 ± 0.04 °C, 56.4 ± 0.4 % r.h. (means ± SE) and L12:D12 reversed photoperiod with scotophase from 7.00 to 19.00 h. For both sexes, newly emerged adults (less than 24 h old) were directly used for microscopic preparations or anaesthetized with CO_2 for the electrophysiological experiments.

Plants

Maize (*Zea mays* L., cv. 511) seeds were provided by Simlaw, Kenya Seeds Company (Nairobi, Kenya). The plants were grown in individual plastic pots (13 cm top diameter and 12 cm high) containing peat, in a greenhouse at ICIPE. The environmental conditions were ca 31/17 °C (day/night) with L12:D12 photoperiod. After three weeks of growth, the plants were placed under the same reversed photoperiod conditions as the insects 48h before use. A gravid female of *B. fusca* was deposited on the plant stem. The portion of the plant where the ovipositor sweep occurred, as described by Calatayud *et al.* (unpublished), was collected for microscopy. A portion of the same plant not touched by the ovipositing female was used as control.

Scanning electron microscopy

Five males and five females and the above portions of stems were fixed over-night in a 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) solution. Then, the insects were dissected to separate foreleg tarsi, antennae and ovipositors. The dissected organs and the stem portions were dehydrated in a graded series of ethanol (70%, 90%, and 100%) and air-dried. The specimens were mounted on stubs with conductive double-side adhesive tape, sputter-coated with gold, and finally examined with a JEOL JSM-T330A SEM.

Silver nitrate staining

The presence of pores in the sensilla on foreleg tarsi, antennae and ovipositors of the 10 individuals were revealed by the penetration of silver nitrate. Entire males and females were stained according to the method of Nayak & Singh (1983) modified as follows: they were first immersed for 1 h in 70% ethanol containing 1 M silver nitrate and then dehydrated in a graded series of ethanol (90% and 100%). After separation of foreleg tarsi, antennae and ovipositor, the organs were cleared in xylene over-night. Then, the samples were mounted in Mountex (Histolab) for light microscope observations.

Electrophysiology

Putative taste sensilla on the tarsi, the antennae and the ovipositor were probed with a tip-recording electrode to determine if the sensilla had chemosensory function. A silver wire inserted into the thorax served as a reference electrode. The sensilla were probed for electrical contact with a capillary electrode filled with 10 mM KCl, which was connected to a TasteProbe (Marion-Poll & Van der Pers 1996). The action potentials were further amplified, filtered (10-2800 Hz), recorded on a computer and analyzed for the presence of action potentials (Marion-Poll 1996).

Data analysis

Statistical tests were performed with Statview software (Abacus Concept, version 5.0, USA). Mann-Whitney U-test was used to compare the length and the number of antennal segments between males and females.

Results and Discussion

Chemosensilla on the antennae

The external morphology of *Busseola fusca* antennae has strong sexual dimorphism (ICIPE Annual Report 1993). Except for the basal and the fourth terminal segments, the male moth has pectinate segments along their flagellum, which are not present on female antennae (fig. 1). Antennae of males were on average 7.6 ± 0.07 mm and were significantly shorter than those of females, which measured 8.3 ± 0.08 mm (mean ± SE, n = 10) (p = 0.0002, Mann-Whitney U-test). The number of segments per flagellum did not vary with sex (62.8 ± 1.7 for males and 64.2 ± 1.0 for females; p = 0.8789, Mann-Whitney U-test). Sensilla styloconica, coeloconica, chaetica and trichoidea observed in *B. fusca* have already been described on the antennae of other noctuids such as *Trichoplusia ni* (Hübner 1802); *Helicoverpa zea* Boddie 1850; *Spodoptera ornithogalli* (Guenée 1852), *Spodoptera*
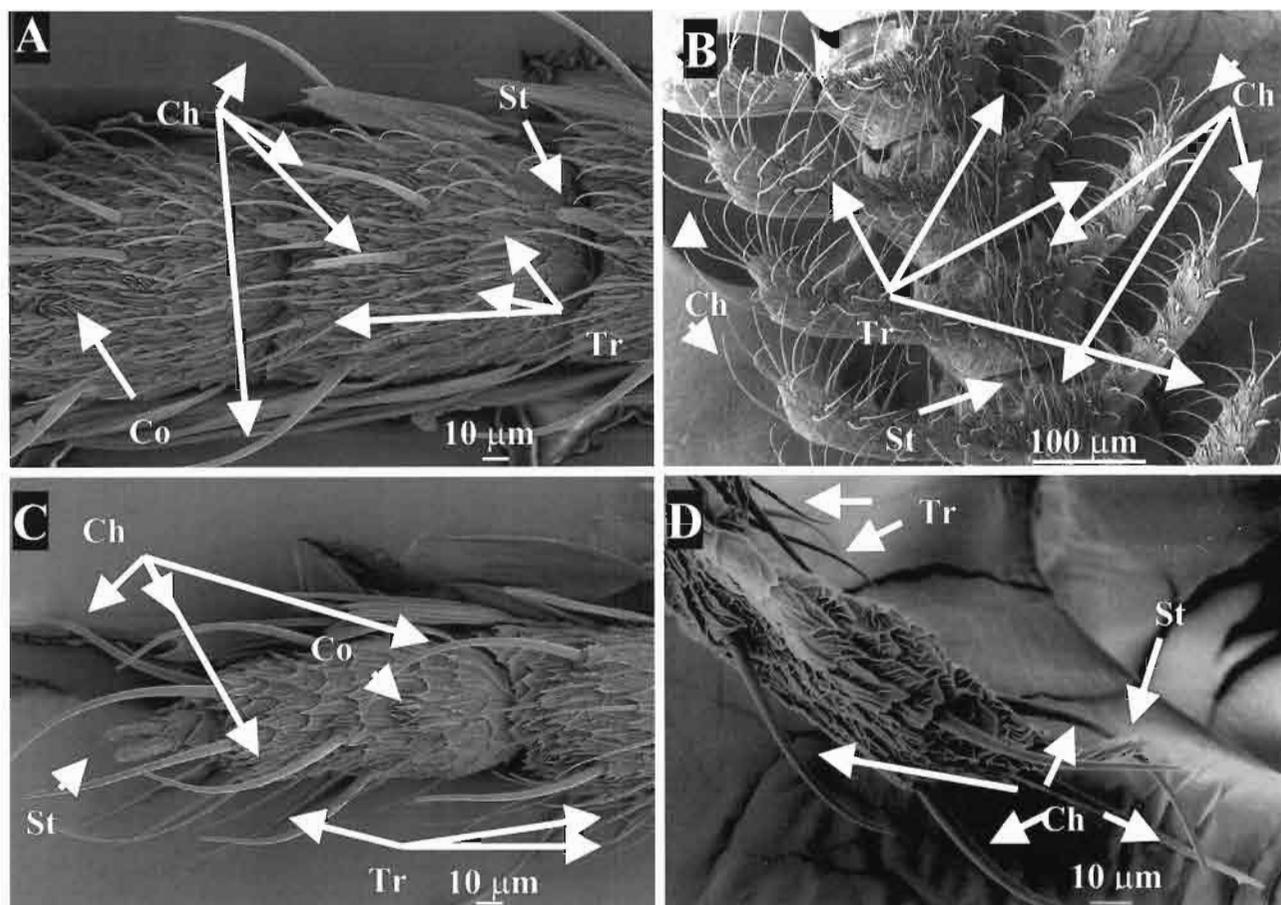
Figure 1

Sensilla types present on the ventral surface of *Busseola fusca* female antenna (A, C) and male (B, D) (A, B: middle segments of the antenna; C, D: apical segment of the antenna) (Ch, sensilla chaetica; Co, sensilla coeloconica; St, sensilla styloconica; Tr, sensilla trichoidea).

exigua (Hübner 1808), *Pseudaletia unipuncta* (Haworth 1809) and *Copitarsia consueta* (Walker 1857) (Jefferson *et al.* 1970; Lavoie & McNeil 1987; Castrejon-Gomez *et al.* 1999).

For both male and female *B. fusca*, one sensillum styloconicum was visible on the terminal part of each antennal segment as well as on the apical segment showing a double structure, which is frequent in noctuids (Castrejon-Gomez *et al.* 1999). These sensilla were argyrophilic or silver stained from their apical part (fig. 2), indicating the presence of an apical pore that suggests contact chemoreceptive function (Jefferson *et al.* 1970). However, this could not be confirmed by electrophysiological recording since these sensilla were not accessible to the electrode.

Sensilla coeloconica were observed on the antennae of male and female *B. fusca*. They also appeared to be argyrophilic. Electrophysiological recording was also not possible. Castrejon-Gomez *et al.* (2003) reported

that these sensilla house olfactory-receptor cells in pyralids, possibly sensitive to volatile compounds.

Sensilla chaetica were observed, distributed along the antennal segments with slight differences between male and female antennae. In females, two longer (length ≈ 80 μm) and two shorter (length ≈ 60 μm) sensilla chaetica were located on the lateral and the medium part of the ventral face of the antennal segment, respectively (fig. 1a), whereas in males two longer (length ≈ 90 μm) chaetica sensilla and a shorter one only (length ≈ 54 μm) were observed (fig. 1b). Females possessed ca seven sensilla chaetica (length ≈ 83 μm) on the terminal antennal segment (fig. 1c), while male moths had six shorter sensilla chaetica (length ≈ 62 μm) (fig. 1d). For both males and females, one sensillum chaeticum (length ≈ 60 μm) was observed on the dorsal part of each segment located between the terminal and the basal segments. For both sexes, all these sensilla were silver-stained, indicating their

porous characteristic (fig. 2). Electrical contact and spike trains were recorded from long and short sensilla chaetica (fig. 3a). Such gustatory sensilla are frequently found on antennae of various species of noctuids and pyralids (Castrejon-Gomez *et al.* 1999; 2003). They have a basal socket, which indicates a bimodal taste/tactile function (Altner *et al.* 1977). During oviposition behaviour, *B. fusca* females walk up and down, touching the plant surface with the apical part

of the antennae (Calatayud *et al.* unpublished) (fig. 8a and b). Since this behaviour step occurred irrespective of the plant status (host and non-host plants) and that gustatory sensilla were evident on the segments close to the terminal part of the antennae, they may play a role in the detection of plant surface chemicals that elicit host plant acceptance by the ovipositing females. For males, their function is still unclear. However, it is possible that during the evolution of the species, they
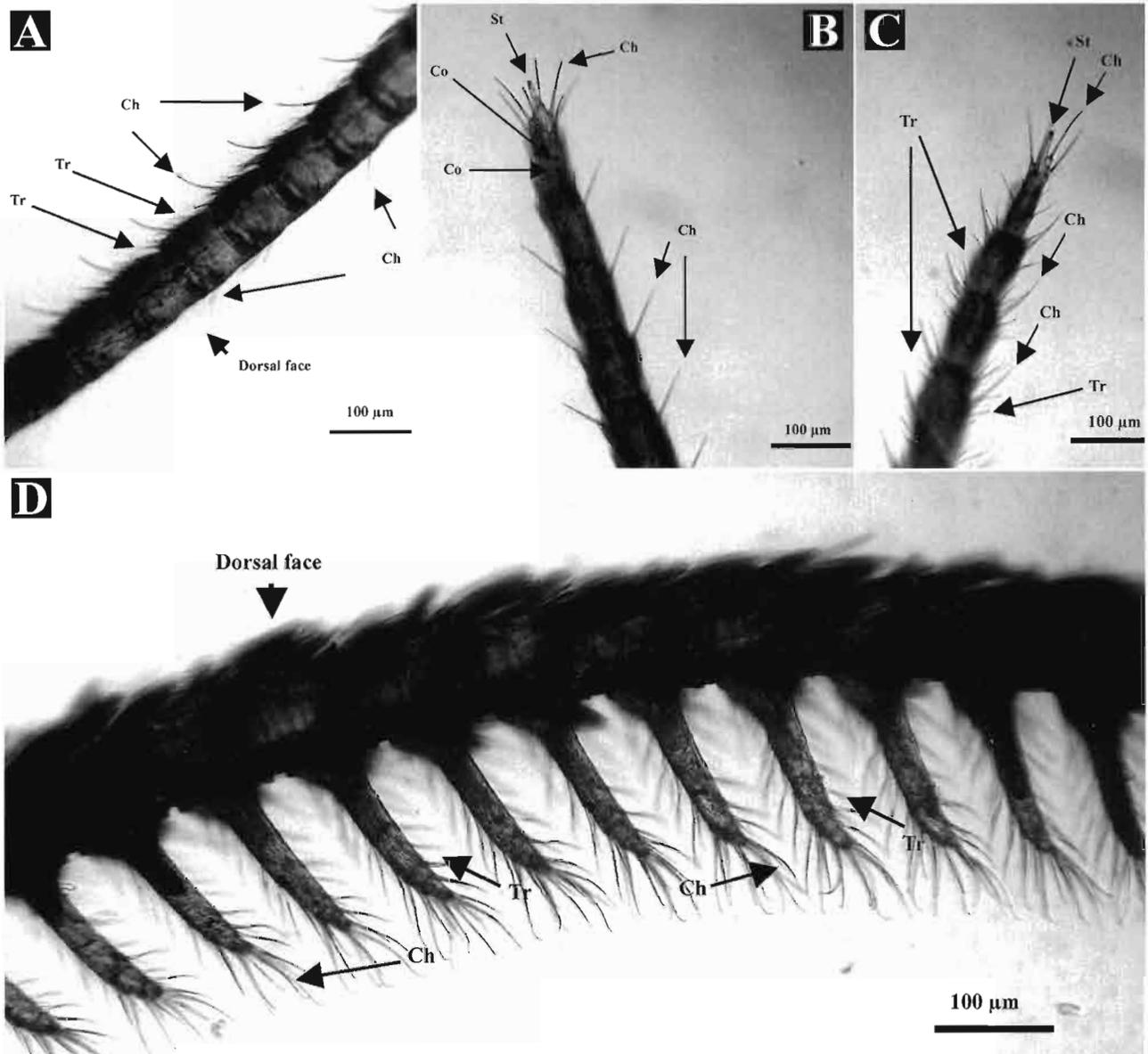
Figure 2
Silver staining impregnation of antennae of female (A, C) and male (B, D) B. fusca (A, B: middle part of the antenna; C, D: terminal part of the antenna). Both types of sensilla, chaetica (Ch) and trichoidea (Tr), were silver stained as well as the sensilla styloconica (St) and the coeloconica (Co) showing black spots distributed along the flagellum.

may have been important if mating took place on the host plant.

Sensilla trichoidea were frequently observed on the ventral face of the antennae. In females, they were 23-40 µm long while in males, the length varied between 38-46 µm on the middle part of the antennal segments and 72-83 µm on the segmental branches (fig. 1). Moreover, there were more numerous on male antennae (97-102 per segment), than on female antennae, that scored 58-61 sensilla per segment. For both sexes, the sensilla number decreases drastically with the distal position of the segment. Only 10 sensilla were count on the terminal segment. All sensilla trichoidea were argyrophilic (fig. 2), indicating that the sensilla shafts are porous. KCl solution evoked action potentials (data not shown) in these sensilla. Sensilla trichoidea are frequently described on the antennae of noctuids and pyralids (Faucheux 1990; Castrejon-Gomez *et al.* 2003) and, because of their multiporous nature, they are putative chemoreceptors for volatile components. The long sensilla trichoidea (72-83 µm in length) found on male antennae, were not present on female antennae. Such long sensilla trichoidea in males are frequently

reported in noctuids and pyralids as dedicated to sex pheromone reception (Faucheux 1990; Castrejon-Gomez *et al.* 1999; 2003). For *B. fusca* females, sensilla trichoidea may play a role in the detection of volatil compounds released by the host plant. Further work remains to be done to validate this hypothesis.

Chemosensilla on the fifth tarsomere of the prothoracic legs

In both *B. fusca* males and females, the fifth tarsomeres of the tarsus are densely covered with scales on the dorsal and lateral sides but not ventrally (fig. 4). The tarsus is prolonged by the pretarsus, which bears two long sclerotized claws, a median arolium, and two lateral pulvilli covered by cuticular ornamental 'microtrichia'. Two types of sensilla chaetica, short ($\approx$ 36-50 µm) and long ($\approx$ 57-71 µm), are distributed ventrally and laterally respectively along the fifth tarsomere (fig. 4). In *B. fusca* males, the fifth tarsomere possesses more sensilla chaetica than in females. They have about 16 sensilla chaetica: five ventral pairs, one latero-ventral pair on the distal part of the tarsomere and two lateral pairs of sensilla (fig. 4b). Females have only 8 sensilla chaetica: two ventral pairs, one latero-ventral pair on
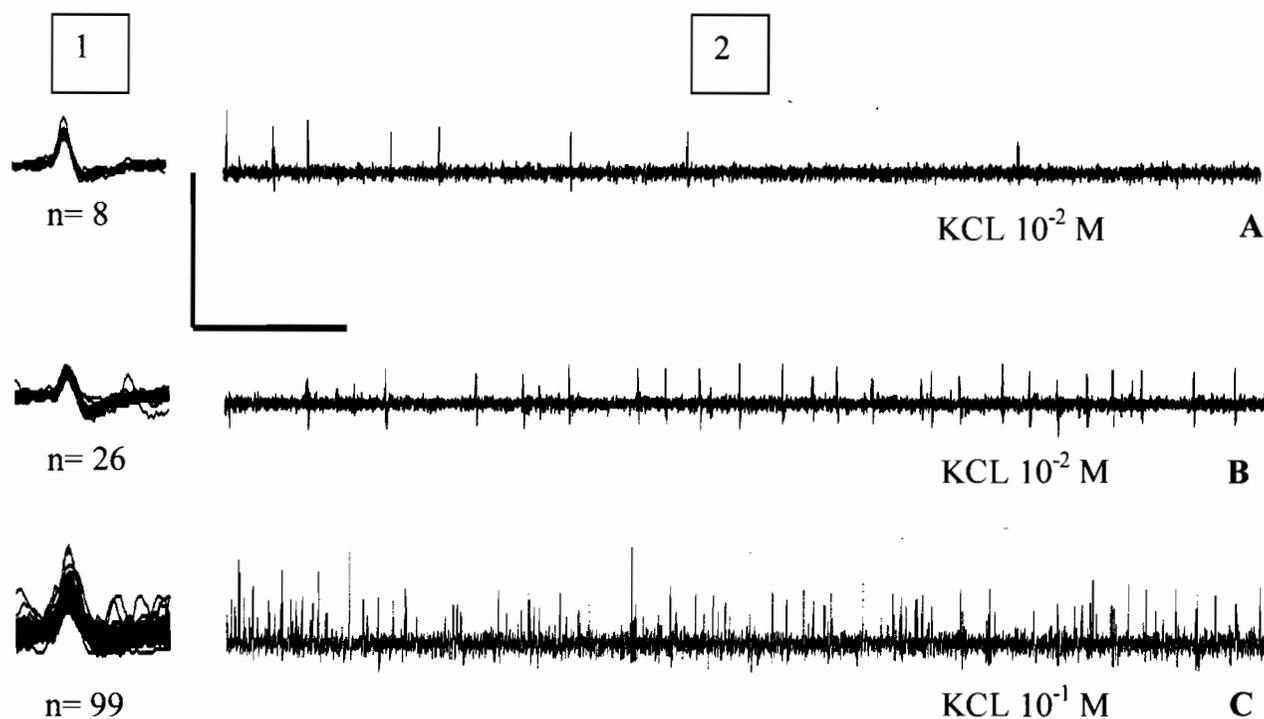

Figure 3
Electrophysiological recordings of action potentials (spikes) obtained after contact with a long lateral chaeticum sensillum of female antennae (A), a long sensillum chaeticum on the fifth tarsomere of a female prothoracic leg (B) and a short sensillum chaeticum located on the ovipositor (C) in response to dilute KCl solution. 1. Spike detected in the recording (6 ms epochs) and superposed to evaluate the distribution of their amplitude and shape; 2. Sample recordings obtained by capping the taste sensilla with a capillary tube containing KCl during 2 s. Vertical bar: 5 mV, except 3 mV for A, horizontal bar: 200 ms.

Figure 6
Tip of a ventral sensillum chaeticum located on the fifth tarsomere of the prothoracic leg of *Busseola fusca* female at high magnification.

Figure 4
Ventral view of the fifth tarsomere and pretarsus of the prothoracic legs of *Busseola fusca* female (A) and male (B). The fifth tarsomere bears short (a) and long (b) sensilla chaetica and the pretarsus bears long (c) and short (d) sensilla chaetica. ARO indicates the central arolium, PUL, a pulvillus; CLAW, a claw; Sc, a scale and Sp, a spine.
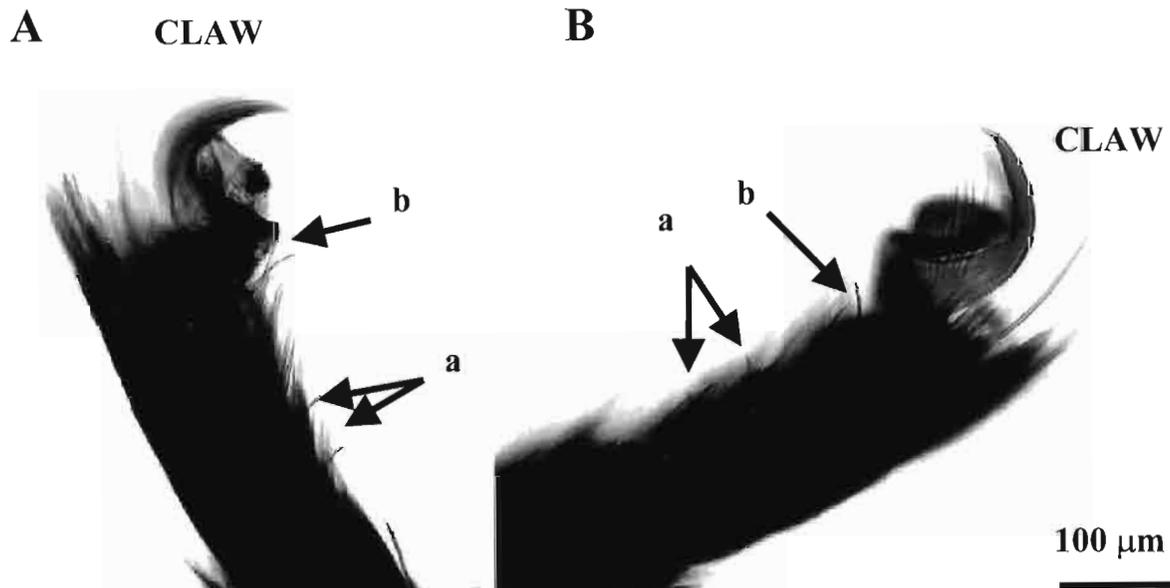
Figure 5
Silver staining impregnation of the fifth tarsomere of *Busseola fusca* prothoracic leg of female (A) and male (B). Short (a) and long (b) sensilla chaetica were silver stained.
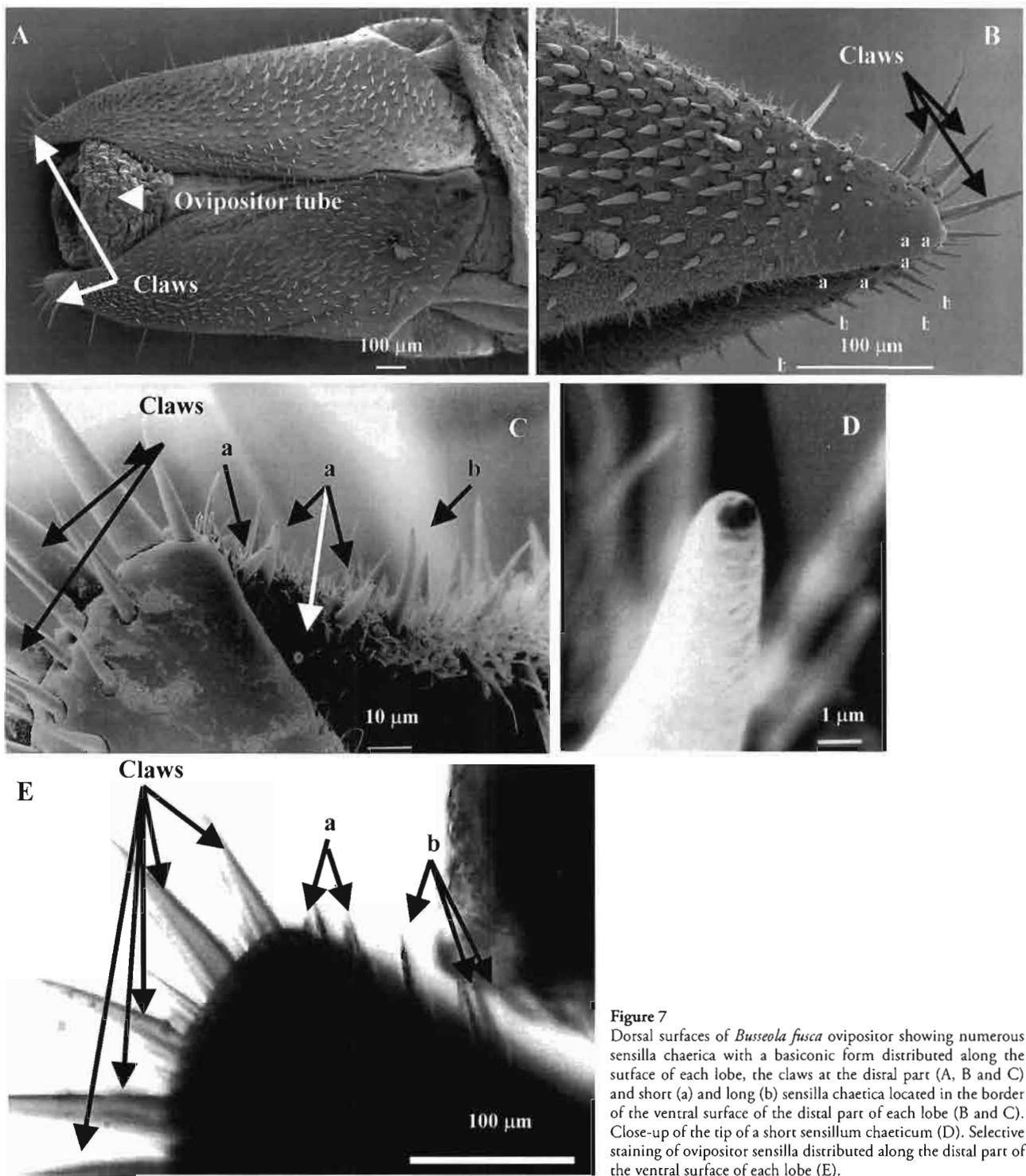
Figure 7
Dorsal surfaces of *Busseola fusca* ovipositor showing numerous sensilla chaetica with a basiconic form distributed along the surface of each lobe, the claws at the distal part (A, B and C) and short (a) and long (b) sensilla chaetica located in the border of the ventral surface of the distal part of each lobe (B and C). Close-up of the tip of a short sensillum chaeticum (D). Selective staining of ovipositor sensilla distributed along the distal part of the ventral surface of each lobe (E).

the distal part of the tarsomere and one lateral pair of sensilla (fig. 4a). All these sensilla are argyrophilic (fig. 5) and bear an apical pore as observed under high magnification (fig. 6). Trains of action potentials

were recorded from these sensilla upon stimulation with KCl (fig. 3b), thus confirming their gustatory function. Taste sensilla on the fifth tarsomere have also been described in pyralids (Waladde 1983; Anderson

409
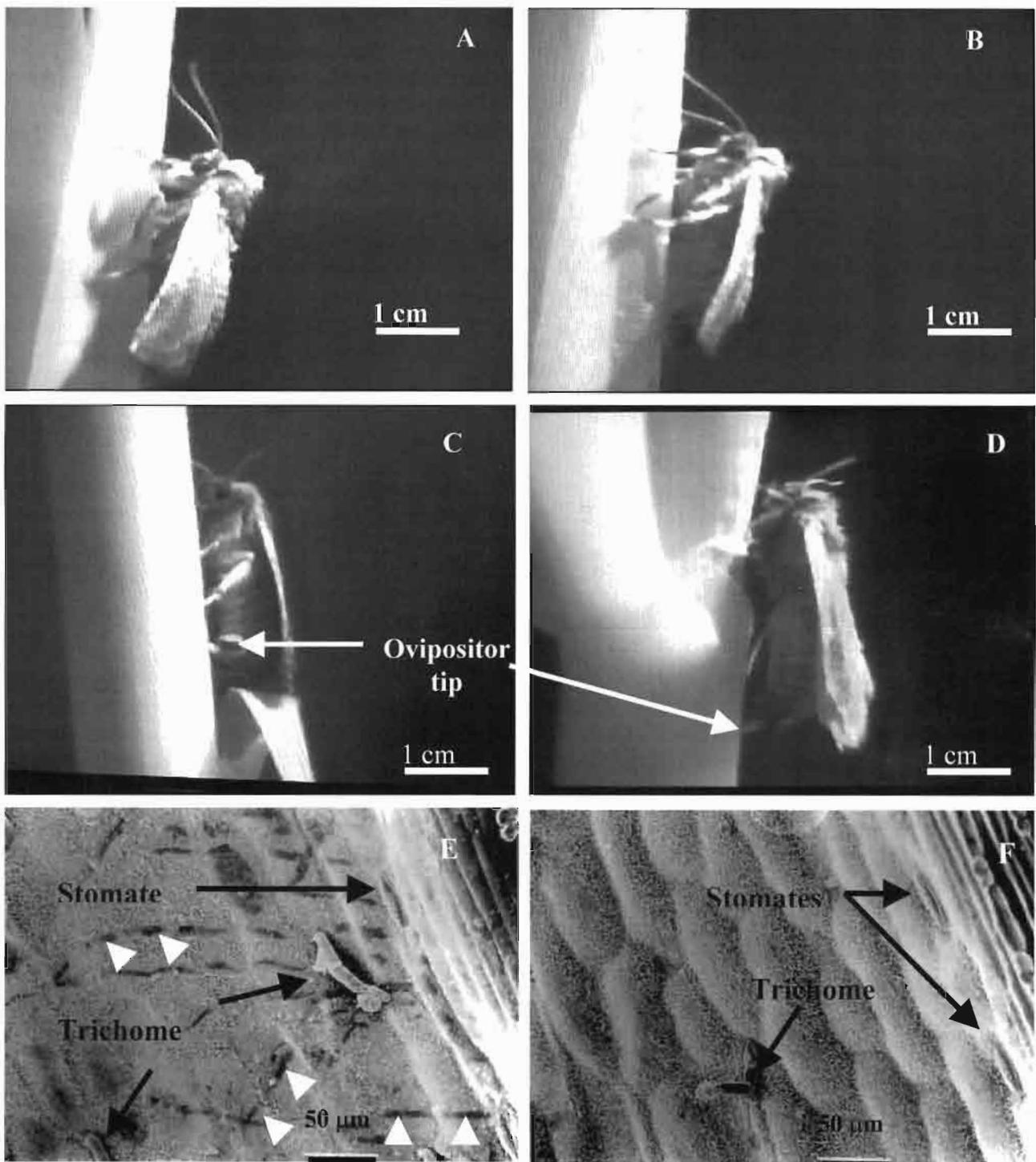
Figure 8
Typical oviposition behaviour in *Busseola fusca*: touching the plant surface with the antennae (A and B) and sweeping with the tip of the ovipositor (C and D). External surface of a maize leaf sheath after been swept by *B. fusca* ovipositor, showing several scratches (white arrowheads) left by the claws of the distal part of the ovipositor (E). External surface of a maize leaf sheath not swept by the ovipositor (=control) (F).

& Hallberg 1990; Marion-Poll *et al.* 1992; Faucheux 1995) but in *B. fusca*, their number is considerably lower. Both female and male pretarsi have two long sensilla chaetica, not argyrophilic. No electrical contact was possible for electrophysiological tip recordings on such sensilla. On the other hand, male pretarsi have an additional argyrophilic sensillum chaeticum, that is located dorsally on the pseudempodium between the two claws and overlapping above the arolium. These were not observed in females. This type of sensilla is common to many moths and has been suggested to have a contact chemo-receptive function (Marion-Poll *et al.* 1992; Maher & Thiery 2004).

Chemosensilla on the ovipositor

The last tergite and sternite of female *B. fusca* form two sclerotised lobes that surround the anal pore and the ovipore (fig. 7a). On the distal part of each lobe seven sclerotized claws are present. Six types of cuticular extensions are distinguishable on the ovipositor with regard to their shape, length and permeability to silver nitrate.

Numerous non-argyrophilic microtrichia are distributed over the lobe surface, similar to the pyralids (Marion-Poll *et al.*, 1992), and can be considered as simple cuticular ornamentations.

About five non-argyrophilic long sensilla chaetica ($\approx$ 150-170 µm) were present on the external border of each lobe. No electrical contact was obtained for electrophysiological tip recordings, suggesting that this sensillum type is not involved in gustative function.

Shorter non-argyrophilic sensilla chaetica ($\approx$ from 19 to 35 µm) are located mostly on the distal part of each lobe surrounding the claws.

Numerous other cone-shape sensilla chaetica measuring between 11 to 65 µm, with the longer ones on the ovipositor dorsal surface were also not stained by silver nitrate.

All the non-argyrophilic sensilla observed above were assumed to have mechanoreceptive function in *B. fusca*, confirming the importance of tactile input during oviposition as described by Calatayud *et al.* (unpublished).

Further six short sensilla chaetica (labelled 'a' in fig. 7b and c) and four long sensilla (labelled 'b' in fig. 7b and c), are argyrophilic and measure about 15 and 26 µm respectively. These were observed on the inner border of the ventral surface of each lobe, close to the anal and ovipositor openings. Several characteristics indicate that these sensilla possess a gustative function. A pore at the tip of each sensillum is clearly visible at high magnification (fig. 7d) and the recorded spike trains are typical of gustatory sensilla (fig. 3c).

The ovipositor of *B. fusca* bears a considerable number of mechanosensory sensilla of different lengths distributed over its surface and only about nine chemosensory sensilla are located within the inner border of the ventral surface of each lobe. These differences in number and distribution may be related to the process of probing the host plant surface for acceptance for oviposition. Prior to oviposition, *B. fusca* females were very sensitive to the shape and structure of the oviposition support (Calatayud *et al.* unpublished). The female insects usually extended the abdomen bending it ventrally, while the terminal abdominal segments protruded from their normal retracted position. This was followed by the behaviour termed as "ovipositor sweep". It included touching the stem surface by the tip of the ovipositor followed by the dorsal extremity making broad lateral movements up and down along the stem (figs. 8c and d). It has been suggested that a combination of tactile and gustatory stimuli from the plant plays a role in the decision-making for host plant acceptance for oviposition. The insects spent much more time sweeping with the ovipositor on the more preferred plant species for oviposition (Calatayud *et al.* unpublished). It is possible that mechanoreceptors enable the female to assess the geometrical/textural configuration of the plant surface to help the placement of eggs (Hallberg & Ahman 1987). Moreover, during the "ovipositor sweep", the claws at the distal part of the ovipositor left small injuries on the plant surface (fig. 8e). They may be deep enough to liberate inner plant cuticular compounds, which are most likely different from those present on the undamaged plant surface. The damaged plant surface may liberate both gustatory and olfactory stimuli that are detected by the ovipositor sensilla. Such a perception activated the appropriate behaviour (acceptance or rejection) depending on the nature of the chemicals.

Conclusion

The identification of different types of chemosensilla on the antennae and ovipositor of *B. fusca* females corroborated observation on oviposition behaviour. While the females walk down the stem of the host plant, they exhibit antennae and ovipositor movements that suggest that these organs are in contact with the stem surface. These movements can be interpreted as probing of the plant surface. The evidence of a sensory equipment allowing chemoreception on both female antennae and ovipositor confirmed that the ovipositing female evaluates the plant before deciding to lay eggs. The results suggest that during probing, the female can access plant chemicals, especially the inner chemicals

presented on the injured part of the plant. The claws on the apical part of the ovipositor lobes injured the stem surface and the gustatory receptors located close to them enable detection of the chemicals by their apical pore. The choice of the suitable host plant is crucial for *B. fusca* since the neonate larvae do not have a strong propensity to migrate onto other nearby plants. Further studies are needed to identify chemical cues released by the damaged plant cuticle, that the antennae and the ovipositor perceive during the antennation and the sweeping behaviour, respectively. In addition, the role of contact chemosensilla on the tarsi remains to be elucidated.

For *B. fusca* males, apart from the possible olfactory function of the antennal trichoidea sensilla that are involved in sex pheromone detection, the role of gustatory sensilla on the antennae and the tarsi remains unclear.

Reference

Altner H. 1977. Insect sensillum specificity and structure: an approach to a new typology, p. 295-303 *in*: **Le Magnen J., MacLeod P. (eds)**, *Olfaction and taste IV*, IRL, London, Washington.

Altner P.J., Sas H., Altner I. 1977. Relationship between structure and function of antennal chemo-, hygro-, and thermo-receptive sensilla in *Periplaneta americana*. *Cell Tissue Research* 176: 389-405.

Anderson P., Hallberg E. 1990. Structure and distribution of tactile and bimodal taste/tactile sensilla on the ovipositor, tarsi and antennae of the flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). *International Journal of Insect Morphology and Embryology* 19: 13-23.

Castrejon-Gomez V.R., Valdez-Carrasco J., Cibrian-Tovar J., Camino-Lavin M., Osorio R. O. 1999. Morphology and distribution of the sense organs on the antennae of *Copitarsia consueta* (Lepidotera: Noctuidae). *Florida Entomologist* 82: 546-555.

Castrejon-Gomez V.R., Nieto G., Valdes J., Castrejon F., Rojas J.C. 2003. The antennal sensilla of *Zamagiria dixolophella* Dyar (Lepidotera: Pyralidae). *Annals of the Entomological Society of America* 96: 672-678.

Faucheux M.J. 1990. External ultrastructure of sensilla on the male and female antennal flagellum of *Noctua pronuba* L. (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France* (n.s.) 26: 173-184.

Faucheux M.J. 1995. Sensilla on the antennae, mouthparts, tarsi and ovipositor of the sunflower moth, *Homoeosoma electellum* (Hulster) (Lepidotera: Pyralidae): a scanning electron microscopic study. *Annales des Sciences naturelles, Zoologie, Paris* 16: 121-136.

Hallberg E., Ahman I. 1987. Sensillar types of the ovipositor of *Diasineura brassica*: structure and relation to oviposition behaviour. *Physiological Entomology* 12: 51-58.

ICIPE Annual Report 1993. *Annual report highlights*. ICIPE, Nairobi, Kenya, 36 p.

Jefferson R.N., Rubin R.E., Mcfarlands S.U., Shorey H.H. 1970. Sex pheromones of noctuid moths, XXII. The external morphology of the antennae of *Trichoplusia ni, Heliothis zea, Prodenia ornithogally,* and *Spodoptera exigua*. *Annals of the Entomological Society of America* 63: 1227-1238.

Kfir R., Overholt W.A., Khan Z.R., Polaszek A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology* 47: 701-731.

Khan Z.R., Pickett J.A., Van Den Berg J., Wadhams L.J., Woodcock C.M. 2000. Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science* 56: 957-962.

Lavoie D.J., McNeil J.N. 1987. Sensilla of the antennal flagellum in *Pseudaletia unipuncta* (Haw.) (Lepidoptera: Noctuidae). *International Journal of Insect Morphology and Embryology* 16: 153-167.

Maher N., Thiery D. 2004. Distribution of chemo- and mechanoreceptors on the tarsi and ovipositor of female European gravepine moth, *Lobesia botrana*. *Entomologia Experimentalis et Applicata* 110: 135-143.

Marion-Poll F. 1996. Display and analysis of electrophysiological data under Windows (TM). *Entomologia Experimentalis et Applicata* 80: 116-119.

Marion-Poll F.C., Guillaumin D., Masson C. 1992. Sexual dimorphism of tarsal receptors and sensory equipment of the ovipositor in the European corn borer, *Ostrinia nubilalis*. *Cell and Tissue Research* 267: 507-518.

Marion-Poll F., Van Der Pers J.N.C. 1996. Un-filtered recordings from insect taste sensilla. *Entomologia Experimentalis et Applicata* 80: 113-115.

Nayak S.V., Singh R.N. 1983. Sensilla on the tarsal segments and mouthparts of adult *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). *International Journal of Insect Morphology and Embryology* 12: 273-291.

Onyango F.O., Ochieng'-Odero J.P.R. 1994. Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis et Applicata* 73: 139-144.

Slifer E.H. 1960. A rapid and sensitive method for identifying permeable areas in the body wall of insects. *Entomological News* 71: 179-182.

Waladde S.M. 1983. Chemoreception of adult stem-borers: tarsal and ovipositor sensilla on *Chilo partellus* and *Eldana saccharina*. *Insect Science and its Application* 4: 159-165.

Zacharuk R.Y., 1985. Antennae and sensilla, p. 1-69 *in*: **Kerkut G.A., Gilbert L.I. (eds)**, *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Pergamon Press, Oxford.

Courtship behaviour of the African Maize Stem Borer: *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) under laboratory conditions

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Abstract. *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is the most important African stem borer damaging maize and sorghum. Chemical mediators play an essential role in all life cycle of this moth, especially for mating recognition and host plant choice. The female sex pheromone, courtship and mating behaviours act on the reproductive isolation within insect populations. *B. fusca* courtship behaviour was studied to decipher each step that could account as a process for reproductive isolation. *B. fusca* males and females presented a very simple and fast courtship behaviour, without any particular events or male pheromone emission.

Résumé. Comportement de cour du foreur africain de graminées : *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) en conditions de laboratoire. *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae) est le plus important ravageur des cultures de maïs et de sorgho en Afrique. L'écologie chimique est essentielle pendant toute la vie de ces Lépidoptères nocturnes et intervient pour la rencontre des partenaires sexuels et le choix de la plante hôte. La phéromone sexuelle produite par la femelle et le comportement précopulatoire sont des facteurs importants du maintien de l'isolement reproducteur au sein des populations de cet insecte. Le comportement de cour de *B. fusca* a été étudié pour préciser chaque événement comportemental, qui pourrait avoir un rôle dans l'isolement reproducteur. Le comportement de cour s'avère être très simple et rapide, sans événement particulier ni émission d'une phéromone mâle.

Keywords: Stem borer, *Busseola fusca*, Noctuidae, Africa, mating and calling behaviours.

Moth reproduction i.e. mate location, courtship behaviour and copulation, mostly relies on the use of chemical signals. Long range behaviour is steered by the female produced sex pheromone perceived by the male that flies upwind to where the female is standing in calling posture. This scheme is, with few exceptions, common in all moths from the more primitive to the more evolved species. In contrast, the characteristic close range behaviour of courtship is very diverse between species and even within the same genus, with no relation between complexity and evolutionary stage. In some species, courting males release chemicals and engage a chemical dialogue with the females, that seems to be involved in sexual selection (Birch *et al.* 1989), whereas some species exhibited a courtship behaviour reduced to copulation attempts. Male produced scents are chemically very diverse (Blum 1987), as well as the

specialized scent structure (Birch & Hefetz 1987).

The specific mate recognition system expressed between males and females (SMRS) is constituted by a complex set of adaptive traits and thus can be considered as an essential element in the evolution of moth populations. Specifications of each SMRS depend in part on the phylogenetic history of each species and on complexity and stability of the environment in which the insects develop. In insects and especially moths, SMRS plays a major role in reproductive isolation and speciation processes. Thus, the chemically disturbed environment produced by sympatric species and interspecific competition has to be taken into account for understand means implemented by our insect to realize its reproductive isolation. Achievement of reproductive isolation is linked to different processes such as the chemistry of the blend, the difference in dial periodicity of sexual behaviour and the possible adult sympatry. At least, when two species share the same pheromone blend and when they behave in sympatry with the same timing in sexual activity, courtship behaviour is the last protection against interspecific copulation.

Busseola fusca (Fuller 1901) (Lepidoptera: Noctuidae) is the most important stem borer species damaging cultivated gramineae: sorghum (*Sorghum bicolor* Moench 1794) and maize (*Zea mays* L. 1753) in Africa (Ratnadass *et al.* 2001). The larvae cause yield losses ranging from region to region from 20% to 80%. *B. fusca* populations have moved from native wild sorghum to cultivated cereal plants and developed a preference for them (Le Rü *et al. in lit.*). As populations can be adapted to the local requirements of the habitat, host plant selection by an insect might partly be explained by the phylogeny (Wyatt 1997).

The objective of this study was to describe qualitatively and quantitatively pre-courtship and courtship behaviours in *B. fusca* on cultivated host plants under laboratory conditions. Such knowledge provides information on the process involved in reproductive isolation and also contributes to improve the trap design adapted to the male landing behaviour.

Materials and Methods

Insect rearing

Pupae originated from the mass rearing unit of the International Centre for Insect Physiology and Ecology (ICIPE-Nairobi-Kenya). Individuals were sexed and each sex was kept in separate close rectangular crystal polystyrene containers (27x12x8 cm) on vermiculite until emergence. Adults were collected daily and males were housed together in boxes as described above. Adult females were placed individually into a cylindrical crystal polystyrene box (ø 3x6 cm) until they were used for experiments when two or three days old. Adults were kept under the following conditions: 25 °C, 85±10% R.H. with a 12:12-h light-dark-reversed photoperiod.
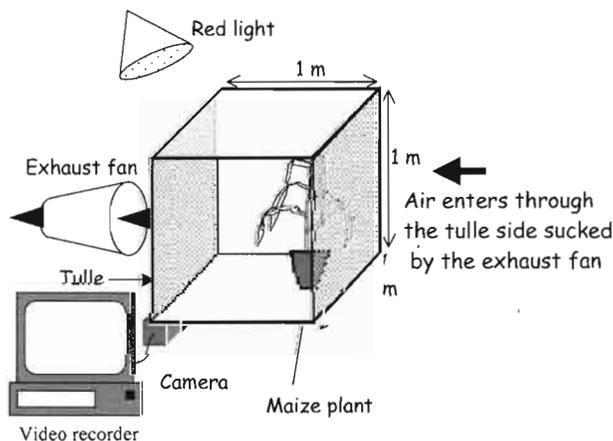
Description and analysis of courtship behaviour

Female calling behaviour is necessary to induce male attraction. The ovipositor of *B. fusca*, like in most moths, is protractible and females initiate calling by extruding the ovipositor and cease calling by retracting it slowly. In our study calling behaviour was defined as 50% of the full ovipositor extension.

According to Calatayud *et al.* (2007), the onset of female calling behaviour is related to the diel periodicity and age. Thus observations of the pre-courtship and courtship behaviours were conducted from the fifth hours after the beginning of the 12h-scotophase. Courtship behaviour was recorded by placing a two to three day-old calling female on a maize plant in a mosquito-net cubic cage (1 m³). Two sides were made of Plexiglas and the other two sides were covered by tulle. A wind fan allowed a constant air flow to pass trough (fig. 1). This airflow set up was necessary to avoid pheromone permeation and for the males to succeed in locating the calling female. Once the female was in calling posture, a male was carefully deposited on the bottom of the cage, downwind from the female. Courtship behaviour was tape recorded (Panasonic AG-7330) until copulation with a Hitachi KP 161 CDD black and white camera equipped with a Nikon objective AF Micro Nikkor 60 mm 1:2.8 D. Each experiment lasted five minutes under 20-25 °C, 45-50% R.H. and red light. The tapes were transcribed in video folders on a computer and analysed with The Observer 5.0 software (Noldus, Wageningen, The Netherlands, 2004) linked to a Psion Workabout.

Results

Female pre-courtship behaviour

Most of the females started to call after the 5th hour of the scotophase. The female remained motionless in calling position. No wing fanning or abdomen puff movements was observed. Females called on the maize plant: 50% were observed on the stem, 35% on the leaf and 15% under the leaf (fig. 2).


Figure 1
Experimental set up for the courtship behaviour study.
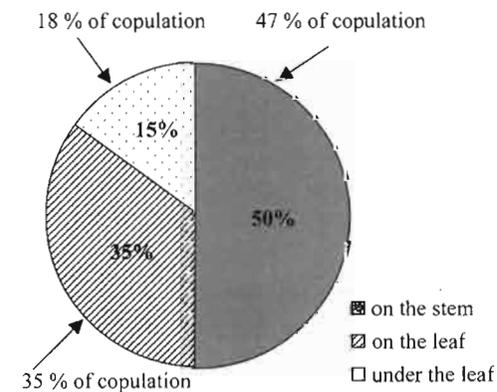
Figure 2
Relation between calling female position and percentage of mating: the percentages on the pie represent the different female positions out of all the tested females. The percentages outside of the pie represent the percentage of copulation out of the total copulation recorded.

Male pre-courtship behaviour

With airflow, moths could sense the dimension and the gradient of the pheromone plume and used this information to steer a course to the source (Cardé & Charlton 1984). As soon as a *B. fusca* male encountered a female pheromone plume, the first behaviours observed were wing fanning, antennae rising up and moving up and down. This step was considered as the activation process before take-off. After taking-off, males flew toward the female in a zigzaging upwind flight in the pheromone plume. This step was clearly an oriented displacement towards the female and lasted an average of 13 seconds.

Courtship behaviour

The observation of 17 complete successful behaviours led us to divide of the courtship behaviour into three steps:

1. The landing. The male landed at different places: either on the stem under the female or on the female side or directly on the female;

2. The attempt to copulate. After landing the male exhibited a wing fanning walk to reach the female and displayed an eversion of the genitalia claspers bearing hair-pencils. Then, the male bent the abdomen to contact the extremity of the female abdomen, trying to hold the female genitalia with the genitalia claspers in copulation attempts;

3. Copulation: the male and female genitalia joined and the male wing fanning ceased. The male spun around 180° staying opposite the female frontal orientation in a tail-to-tail posture typical in Lepidoptera mating. When copulation was successful, male and female remained linked for *ca* three hours.

66% of experimented pairs (n = 26) copulated and the courtship behaviour from the take-off to the contact between male and female genitalia lasted an average of 19.73 seconds (sem ± 19,75). 46% of the males landed on the plant underneath the female and then walked while wing fanning to reach the female side; 27% landed on the side near the female and 27% on the female (fig. 3). Landing underneath the female leads to 48% copulation success whereas 28% and 24% (P = 1.52) success rates were recorded for landing on the female side or directly on the female respectively. When the male landed on the female, calling behaviour stopped and the male could not again locate the female.

Observed unsuccessful copulations (n = 9) were due to female rejection (n = 5), female escaping (n = 2) or a cease of calling (n = 2).

Copulation percentages varied according to the site of calling behaviour: 47% of mating occurred when females called on the stem, 35% when on the leaf and 18% when under the leaf.

Discussion

The *Busseola fusca* courtship behaviour is relatively short and simple. The pheromone modulates the male upwind flight to locate the female. Once the male encounters the female pheromone plume, it usually lands on the stem underneath the female. Despite the fact that our results are not significant certainly due to low number of experiments, it appears that landing on the stem underneath the female is the most efficient for successful copulation and must be taken into account for optimizing the design of the pheromone trap in a way that the males can land underneath the pheromone source and then walk up toward it. Sometimes, the male lands brutally on the female which exhibits a rejection motion and stops calling. Female rejection remains the main reason for unsuccessful mating. In most Lepidoptera, the mate choice is the decision of the females (Zagatti & Castel 1987).

B. fusca males do not present a highly elaborated mating behaviour contrary to other noctuid species such as *Mamestra brassicae* L. 1758, which exhibit a sophisticated mating behaviour with the release of a male sex pheromone produced by the abdominal brushes acting as an attractant for the calling female (Birch *et al.* 1989; Jacquin *et al.* 1991; Noldus & Potting 1989). Male pheromone release is necessary for copulation success and increases female recognition and acceptance at short range. The female are supposed to choose the courting male releasing the higher quantity of male pheromone and a sort of "sexual selection" based on female choice and male pheromone occurs (Frérot unpublished data).
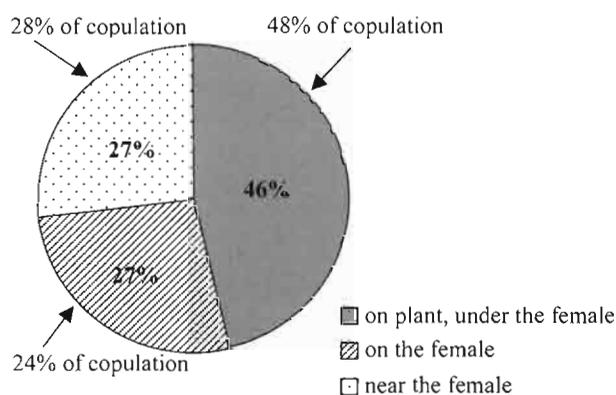

Figure 3
Relation between landing places and percentage of mating: the percentages on the pie represent the different male landing places out of all male tested. The percentages outside of the pie represent the percentage of copulation out of the total copulation recorded.

Clasper hair pencils of *B. fusca* males do not seem to release any sex pheromone. *B. fusca* females seem to select the males on their landing behaviour and to reject copulation, using two means: escape or stop calling. Sexual selection plays an essential role in most of Lepidoptera which copulate only once; only the most efficient moths will copulate. *B. fusca* is a polyandrous species (Calatayud *et al.* unpublished data) and this may account for no mate selection in this species. Thereby, very simple *B. fusca* courtship behaviour and the apparent absence of sexual selection lead to draw some hypothesis. With a sex-ratio favouring males (Ratnadass *et al.* 2001) and with polyandrous females, a mate choice by the female would be a waste of energy and would be detrimental for the species fitness. Sperm selection could be another way for intra specific selection but no information is available on the occurrence of such a process in *B. fusca* female egg fertilization.

The entire pheromone blend functions as a species-specific unitary signal for long range attraction, courtship and copulation (Linn *et al.* 1984). In the case of *B. fusca*, the female sex pheromone is a mixture of three compounds: the (Z)-11-tetradecenyl acetate (main), the (E)-11-tetradecenyl acetate and (Z)-9-tetradecenyl acetate (minors), 70:15:15% respectively (Nesbitt *et al.* 1980; Hall *et al.* 1981). Diversity of moth species developing on maize and sorghum crops in Kenya is reduced. The other main populations developing on these plants are *Sesamia calamistis* Hampson 1810 (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe 1885) (Lepidoptera: Crambidae), which produce a sex pheromone whose main components are characterized by 16 carbons (www-pherolist.slu.se) preventing interspecies attraction.

Many stem borers are associated with their host plants. Phytophagous insects often meet, court and mate on plants (Landolt & Phillips 1997). To date, how host plants influence *B. fusca* SMRS remains unresolved. Maize plants in our study allow the female to be in a good calling position and the male to spin after copulation. Does the plant only represent a calling stand or does it affect sexual behaviour remains to be answered.

References

Birch M.C., Hefetz A. 1987. Extrusible organs in male moths and their role in courtship behavior. *Bulletin Entomological Society of America* 33: 222-229.

Birch M. C., Lucas P., White P.R. 1989. The courtship behaviour of the cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), and the role of male hair-pencils. *Journal of Insect Behavior* 2: 227-239.

Blum M.S. 1987. Biosynthesis of arthropod exocrine compounds. *Annual Review of Entomolomology* 32: 381-413.

Calatayud P.-A., Guénégo H., Le Rü B., Silvain J.F., Frérot B. 2007. Temporal patterns of emergence, calling and oviposition of the maize stem borer, *Busseola fusca* (Lepidoptera: Noctuidae). *Annales de la Société entomologique de France (N.S.)* 43(1): (*in press*)

Cardé R.T., Charlton R.F. 1984. Olfactory Sexual Communication in Lepidoptera: Strategy, Sensitivity and Selectivity, p. 241-265 *in:* T. Lewis (ed.), *Insect Communication*. Academic Press, London.

Hall D.R., Beevor P.S., Cork A., Lester R., Nesbitt B.F., Nyirenda G.K.C, Phiri D.D.N., Blair B.W., Tannock J. 1981. The female sex pheromone of the maize stalk-borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae): identification and initial field trials. *Zimbabwe Journal of Agricultural Research* 19: 111-122.

Jacquin E., Nagnan P., Frérot B. 1991. Identification of hairpencil secretion from male *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) and electroantennogram studies. *Journal of Chemical Ecology* 17: 239-246.

Landolt P. J., Phillips T.W. 1997. Host plant influences on sex pheromone behavior of phytophagous insects. *Annual Review of Entomology* 42: 371-391.

Linn C. E., Bjostad L.B., Du J.W., Roelofs W.L. 1984. Redundancy in a chemical signal: behavioral responses of male *Trichoplusia ni* to a 6-component sex pheromone blend. *Journal of Chemical Ecology* 10: 1635-1658.

Nesbitt B. F., Beevor P.S., Cork A., Hall D.R., Lester R., Blair B.W., Tannock J. 1980. Identification of the female sex pheromone of the maize stalk borer, *Busseola fusca*: a preliminary note. *Tropical Pest Management* 26: 327.

Noldus L.P.J.J., Potting R.P.J. 1989. Calling Behavior of *Mamestra brassicae*: Effect of Age and Photoperiod. *Entomologia Experimentalis et Applicata* 3: 61-71.

Ratnadass A., Traore T., Sylla M., Diarra D. 2001. Improved techniques for mass-rearing *Busseola fusca* (Lepidoptera: Noctuidae) on an artificial diet. *African Entomology* 9: 167-175.

Wyatt T. D. 1997. Methods in studying insect behaviour, p. 27-56 *in:* Dent D.R., Walton M.P. (eds.), *Methods in Ecological and Agricultural Entomology*. CABI, Wallingford.

Zagatti P., Castel Y. 1987. Courtship behaviour of the false codling moth, *Cryptophlebia leucotreta* (Meyrick): androconial display and mating success (Lepidoptera, Tortricidae). *Annales de la Société Entomologique de France (N.S)* 23: 113-123.

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Trichogramma bournieri Pintureau & Babault (Hymenoptera: Trichogrammatidae) and *Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae) in sugarcane in Mozambique: A new association

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Abstract. *Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae), a sugarcane stalk borer indigenous to South East Asia, and the nearby Indonesian Islands, was identified from African sugarcane in Mozambique in 1999. Prior to a classical biocontrol programme being implemented against it, intensive pre-release surveys for the presence of any indigenous natural enemies on life stages of the borer were completed. Negligible parasitism of larval and pupal stages was recorded. In contrast, egg batches found were heavily parasitised. Parasitoid adults emerging from the eggs were found to be only the indigenous *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae). Aspects of the impact of *T. bournieri* on *C. sacchariphagus* eggs in Mozambican sugarcane are presented, and the potential of using this egg parasitoid against *C. sacchariphagus* in an augmentation biocontrol programme is discussed.

Résumé. *Trichogramma bournieri* Pintureau & Babault (Hymenoptera : Trichogrammatidae) et *Chilo sacchariphagus* Bojer (Lepidoptera : Crambidae) de la canne à sucre au Mozambique, une nouvelle association. Le foreur de la canne à sucre, *Chilo sacchariphagus* Bojer, natif du Sud Est de l'Asie et du pourtour des îles indonésiennes, a été identifié au Mozambique en 1999 sur la canne à sucre. Avant l'utilisation d'une lutte biologique classique, une surveillance préliminaire de la présence d'ennemis naturels indigènes des stades de développement du foreur a été entreprise. Un parasitisme larvaire et pupal négligeable a été enregistré. En revanche, les oeufs ont été fortement parasités. Les parasitoïdes adultes qui ont émergé des oeufs ont été identifiés comme étant *Trichogramma bournieri* Pintureau & Babault, la seule espèce indigène rencontrée. Quelques aspects de l'impact de *T. bournieri* sur les oeufs de *C. sacchariphagus* pour le contrôle de ce foreur sur canne à sucre au Mozambique sont présentés, et l'utilisation de tels parasitoïdes dans un contrôle biologique est discutée.

Keywords: New association, egg parasitoid, sugarcane, *Trichogramma*, *Chilo*.

The first exotic stalk borer to be found in very high numbers in sub-saharan African sugarcane was identified by Way & Turner (1999) as *Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae). It was collected by these authors from Acucareira de Mozambique (A de M), Mafambisse (19°20'S34°10'E) in Mozambique, although its presence on this sugar estate was reported in unpublished reports as early as 1989 (van Rensburg *et al.* 1989). In 2001 a further confirmation at a new locality in Mozambique was obtained from Companhia de Sena (18°17'S 35°57'E 6-11 m), at Marromeu on the Zambezi River (Conlong & Goebel 2002). Thus far it has not been found in any of the other sugar estates in Mozambique nor in African countries bordering Mozambique. It

is thus absolutely necessary that control measures be implemented on these two known populations of *C. sacchariphagus* in Africa, so that further incursion, and build up can be prevented, and the threat to African sugarcane production be minimised. The extremely rapid spread of *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), since it was first found in maize in Malawi in 1930 (Tams 1932) and resultant heavy crop losses it has caused since, serves as a current reminder of what an uncontrolled exotic stalk boring pest of graminaceous crops can do in Africa (Overholt *et al.* 1994).

In 2000, the management of A de M requested that a biological control programme be implemented against *C. sacchariphagus* on their estate, and in 2001, the management of Sena requested the same to be completed on their estate. Conlong & Goebel (2002) report on the first field surveys completed at A de M, the impact of *C. sacchariphagus* on their cane, the impact of indigenous parasitoids found

at that time on the *C. sacchariphagus* population, and the results of the first releases of *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) against *C. sacchariphagus* pupae. At that time, minimal indigenous parasitism of larval and pupal life stages of *C. sacchariphagus* was found. However, egg parasitism was abundant. Parasitoids emerging from parasitised egg batches were sent to Dr E. Tabone, INRA, Antibes, France (Entomology and Biological Control Unit), who identified them using morphological characteristics as *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae). Voucher specimens are housed at the Natural History Museum in Paris, France (Conlong & Goebel 2002)

Trichogramma bournieri was reared from *C. partellus* eggs collected in 1982 from maize in Djomani on the island of Ngazidja in the Comores (Bournier 1993). Bonhof (2000) collected the same species from *C. partellus* eggs from maize in coastal Kenya. Here egg parasitism was regarded as the most important mortality factor of eggs, above predation. Although the egg parasitoid was not identified, Gonçalves (1970) recorded 60% egg parasitism on *C. partellus* eggs in Mozambique. Cugala *et al.* (2001) similarly recorded high egg parasitism by an unidentified egg parasitoid in his surveys in Mozambique, which have recently been confirmed as *T. bournieri* by INRA. It is thus regarded as an African species (Haile *et al.* 2002).

It is thus apparent that the egg parasitoid niche is not void, being filled at this stage by only one species of parasitoid, *T. bournieri*. Subsequent to these early reports, a number of visits have been made to the two sugar estates in Mozambique, and more quantitative surveys have been made of the presence of *T. bournieri* on *C. sacchariphagus*. These are the subject of this paper.

Materials and Methods

The life cycle and biology of *Chilo sacchariphagus* is well known and has been described by Williams (1983), Cheng (1994), Kuniata (1994), Way & Turner (1999) and Goebel (1999). Of importance to this paper is that the female *C. sacchariphagus* oviposits predominantly on abaxial and adaxial surfaces of green leaf blades. The eggs are thus not cryptically hidden.

Three approaches were taken to collect eggs. In sugarcane younger than six months, random searched were completed by moving slowly along sugarcane rows, carefully inspecting all green leaf foliage for egg batches. When batches were found, they were carefully removed by excising the piece of leaf it was attached to, and placing the egg batch and piece of leaf into empty 30 ml plastic vials with lids with very fine gauze to prevent parasitoid escape. The vials were labelled with date of collection, field number and variety of sugarcane. In older and taller sugarcane, identified fields were surveyed at two corners. A maximum of one hundred stalks were taken at each corner by positioning

four field inspectors 25 steps apart along the field margin. The inspectors were asked to collect a stalk of cane every five steps along a row until they had collected 25. This process sampled approximately one hectare of sugarcane. Harvested stalks were brought to the field margin, and the green leaf material carefully searched for egg batches before the stalk was dissected for larval and pupal assessment and damage (Conlong & Goebel 2002). If egg batches were found, they were taken and processed as described above. These results gave some information on the extent of *Chilo sacchariphagus* oviposition in the field, and the effectiveness of *Trichogramma bournieri* foraging. A third method of sampling was to inspect the green foliage of older sugarcane along field margins while the field team were taking the stalk samples. The first and third methods of collecting were accomplished in order to collect as many parasitised egg batches as possible, so that any egg parasitoid species assemblage could be detected once the parasitoids had emerged from the parasitised eggs. In all collections, egg batches were identified as unparasitised and parasitised. During the final few collections, the positions of the egg batches on the leaf were identified to determine if there was any stratification in *T. bournieri* foraging. The green leaves were numbered, with number 1 being the terminal newly formed leaf, 2 being the next youngest, and so on until the first older dead leaves were encountered. In addition to the number, the position of the batch on the leaf was recorded, i.e. top or bottom of leaf, and also if the batch was on the blade itself, or the midrib of the leaf.

Vials containing *C. sacchariphagus* eggs were sent to the South African Plant Protection Research Institute Quarantine Laboratory where they were screened for parasitoid emergence. All parasitoid adults that emerged, were preserved in 95% ethyl alcohol, and were sent to the INRA laboratories in Antibes, France for identification. The empty egg batches were then sent to South African Sugarcane Research Institute (SASRI) for counting.

Those parasitoid adults still living on arrival, and emerging at the SA government Quarantine laboratories were offered *Chilo partellus, Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *Galleria mellonella* L. (Lepidoptera: Pyralidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. They accepted all species except *E. kuehniella* as hosts, although *C. partellus* eggs were not as heavily parasitised as those of *H. armigera* and *G. mellonella*. A laboratory colony was established on

Table 1. Parasitism of *Chilo sacchariphagus* egg batches by *Trichogramma bournieri* from field margin surveys at Açucareira de Mozambique (A de M), from October 2001 until May 2003, and at Sena sugar estate from April 2002 to May 2003.

Sugar Estate	Survey Date	Survey Time (Man hours)	Number of egg batches found			
			Parasitised	Unparasitised	Total	% Parasited
A de M	Oct 2001	72	0	4	4	0
	Apr 2002	12	30	4	34	88.2
	Jun 2002	5	19	1	20	95.0
	Oct 2002	7	0	1	1	0
	May 2003	9	32	1	33	97.0
Sena	Apr 2002	12	230	10	240	95.8
	Jun 2002	7	32	1	33	97.0
	Oct 2002	7	0	6	6	0
	May 2003	14	77	7	84	91.7

these factitious hosts at the quarantine laboratories. In addition, material was sent to SASRI, but a colony could not be established on *Eldana saccharina* Walker (Lepidoptera: Pyralidae).

Although egg parasitoids were first found in March and June 2001 at A de M sugar estate, parasitised egg batches were just collected so that parasitoid species determinations could be completed (Conlong & Goebel, 2002). More quantitative surveys were completed at A de M during subsequent visits in October 2001, April, June and October 2002, and May 2003. Because Sena estate requested help a year later the A de M, more detailed surveys commenced immediately regular visits commenced in April, June and October 2002, and May 2003. The most recent surveys at both estates were completed in April/ May 2005.

Results

Extent of Trichogramma bournieri *parasitism of* Chilo sacchariphagus *egg batches*

Table 1 shows that *T. bournieri* parasitism in early spring (October) was very low, with very few *C. sacchariphagus* egg batches found, and none parasitised. However, in mid-winter (May, June) parasitism of egg batches found during field margin surveys increased to 97%.

In the more structured surveys, egg batches were collected from sugarcane stalks taken during stratified random surveys of corners of sugarcane fields. This method of survey gave an idea of the natural oviposition taking place in the section of field surveyed, as well as an idea of the natural parasitism of the egg batches by indigenous egg parasitoids. The results are given from these surveys at Sena Sugar estate (tab. 2), as this estate had a higher population of *C. sacchariphagus*, as evidenced from the egg surveys completed (tab. 1).

The latter method of assessment is a more accurate measure of *C. sacchariphagus* natural oviposition patterns, and subsequent parasitism by *T. bournieri*. Field edge scouting for egg batches is biased towards collecting parasitised batches, as these were always black, and stand out against the green leaf blades. However, some unparasitised eggs were found, especially those close to eclosion. These had a reddish-brown colour, which also stood out against the green leaf sheath. Freshly laid eggs are always creamy white in colour, and blend in with the green leaf blade and its whiter midrib, and are thus harder to see. The pattern of parasitism is still similar, with low parasitism recorded in October, and highest parasitism in Autumn/Winter.

Within-batch parasitism by *Trichogramma bournieri*

At both sugar estates, when parasitised batches of eggs were found, it appeared that all the eggs in the batches, whether comprised of few eggs or many, were

parasitised. Table 3 reveals that this was the case. The mean *C. sacchariphagus* egg number per batch, and the minimum and maximum numbers of eggs per batch are also given.

While counting parasitised eggs, it was noticed that a number of the parasitised eggs had more than one adult parasitoid exit hole. This indicated that more than one egg parasitoid could develop per egg. A random sample of the parasitised egg batches was counted to determine the extent of multiple parasitism (recorded as number of exit holes per egg) of the eggs in the batches. Table 4 summarises the observations.

Of the eggs in the egg batches counted which had adult parasitoid exit holes, 42% had one adult emerging per egg, 22.4% two adults emerging, and only 1% 3 adults emerging. It thus seems that two parasitoid adults can happily develop in a single *C. sacchariphagus* egg, but more generally only one develops per egg, and very rarely three. This needs to be confirmed in more detailed laboratory and field studies though.

Position of *Chilo sacchariphagus* egg batches on sugarcane, and ability of *Trichogramma bournieri* to find them

At the time of the last visit in April/May 2005, when egg batches were found on the green leaves of sugarcane when scouting field margins, their exact

Table 2. Parasitism of *Chilo sacchariphagus* egg batches by *Trichogramma bournieri* from stratified random stalk surveys at Sena sugar estate during 2002.

Survey Date	Total Stalks Surveyed	Number of Egg Batches				Batches / 100 stalks
		Parasitised	Unparasitised	Total	% Parasited	
Apr 02	1250	34	20	54	63.0	4.3
Jun 02	660	6	6	12	50.0	1.8
Oct 02	660	0	0	0	0	0

Table 3. Parasitism by *Trichogramma bournieri* within *Chilo sacchariphagus* egg batches found parasitised at both sugar estates in April 2002 (SD = Standard Deviation).

Sugar Estate	Number Batches	Eggs per Batch				
		Max	Min	Mean±SD	Mean parasit.±SD	Mean % parasited ±SD
A de M	56	44	7	22.86±7.70	19.07±6.22	88.53±22.82
Sena	177	56	9	23.50±8.43	22.42±7.66	97.11±12.81

Table 4. Multiple parasitism in egg batches (measured as number of adult parasitoid exit holes) found parasitised at Sena Sugar Estate.

No. egg batches counted	Mean no. eggs per egg batch±SD	Mean % eggs with parasitoid adult exit holes		
		1 exit hole ±SD	2 exit holes ±SD	3 exit holes ±SD
51	20.9±7.4	42.0±19.2	22.4±11.0	1.0±3.2

position was recorded (as outlined in the Materials and Methods section). Table 5 summarises the findings. At the time of these surveys, only one unparasitised egg batch was found (on the blade of the top surface of the 7th leaf), so the results given reflect only the position of parasitised egg batches.

Although the same period of time was spent scouting fields at A de M and Sena, it is apparent from the Grand total batches of eggs collected that populations of *C. sacchariphagus* were lower at the former compared to the latter estate (35 and 103 batches respectively; tab. 5). Eggs were found on the second to tenth leaves at A de M, and second to twelfth leaves at Sena. The fifth and sixth leaves had the most total egg batches found at both estates. No eggs were found on the youngest first leaf, although a few batches were found on the second and third leaves at both estates. Numbers of egg batches found also tailed off towards the oldest leaves (tab. 5).

The total number of batches laid on the top, or upper surface of the leaves was between 56 and 66% greater than those laid on the lower surfaces of the green leaves at A de M and Sena respectively (tab. 5). Of the batches laid on the top surface of the leaves, the majority were laid on the leaf blade rather than on the leaf midrib (19 vs 7 at A de M and 51 vs 15 at Sena; tab. 5), whereas they were more evenly distributed between the two locations when laid on the under surface of the leaf (5 and 4 at A de M and 21 and 16 at Sena; tab. 5).

In general, oviposition decreased on the underside of leaf blades as the leaves got older, which was in contrast to oviposition on the top surface, where more batches were found on the older leaves, especially at Sena, where the population of *C. sacchariphagus* was larger (tab. 5).

Discussion

Because parasitised eggs are black, they are much easier to see against the green leaf blade than unparasitised eggs, which are creamy in colour. This method of scouting thus biases the sample towards finding parasitised eggs. However, the initial aim of the scouting was to find as many parasitised eggs as possible, so that the egg parasitoid species complex could be determined. This is important to know before a mass release programme is initiated with any one parasitoid. The most effective indigenous egg parasitoid

Table 5. Number of batches of eggs on leaves of sugarcane at both sugar estates on favoured oviposition sites of *Chilo sacchariphagus* and found by *Trichogramma bournieri* (1-youngest green leaf; 13 oldest green leaf).

Sugar Estate	Leaf Number	Bottom Leaf Surface Blade	Top Leaf Surface Midrib	Total	Blade	Midrib	Total	GRAND TOTAL
A de M	2	0	0	0	1	0	1	1
	3	1	0	1	1	0	1	2
	4	0	2	2	2	1	3	5
	5	0	0	0	3	1	4	4
	6	1	2	3	4	4	8	11
	7	1	0	1	2	1	3	4
	8	1	0	1	2	0	2	3
	9	1	0	1	2	0	2	3
	10	0	0	0	2	0	2	2
	TOTAL	5	4	9	19	7	26	35
Sena	2	2	0	3	2	1	3	5
	3	1	1	2	4	0	4	6
	4	4	1	5	2	1	3	8
	5	7	4	11	5	3	8	19
	6	1	2	3	8	2	10	13
	7	2	2	4	2	1	2	7
	8	2	2	4	7	1	8	12
	9	2	2	4	9	1	10	14
	10	0	1	1	10	3	13	14
	11	0	1	1	1	1	2	3
	12	0	0	0	1	1	2	2
	TOTAL	21	16	37	51	15	66	103

species should be chosen as the mass-rearing candidate (Smith 1996), so that the local species composition is not altered to any large degree. In these sugarcane estates, the only egg parasitoid found has been *Trichogramma bournieri*, so there are no competition effects to consider should an augmentation biocontrol programme be considered using this species against *Chilo sacchariphagus*.

Accepting the fact that scouting for egg batches will be biased towards finding parasitised eggs, it was still apparent that there is a *C. sacchariphagus* cycle, and a *T. bournieri* cycle related to that at both estates. Populations of *T. bournieri* were at their lowest in October in the years sampled. This would thus be a time when augmentation of the wild population could be done with laboratory-reared individuals. This approach has been followed in Reunion, using *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), with good results (Goebel *et al.* 2001; Soula *et al.* 2003). For control of *C. sacchariphagus* in Mozambique, however, it would be best to augment populations of *T. bournieri* From September through to December each year.

The current extent of field parasitism as measured in the stratified random sampling approach at the sugar estates in Mozambique is higher than what was found in coastal Kenya on *Chilo partellus* egg batches in maize (Bonhof 2000). Haile *et al.* (2002) reported it as the main egg parasitoid of *C. partellus* in Mbita, Kenya, on the shores of Lake Victoria, but did not give parasitism rates found. This indicates that *T. bournieri* attacking *C. sacchariphagus* in sugarcane is a good strain, with high parasitism levels recorded, and thus probably well suited to its host- a desirable character for a species being considered for augmentation (Smith 1996, Haile *et al.* 2002).

Another good attribute of *T. bournieri* is that when a female encounters an egg batch, it generally parasitises the whole batch, thus allowing no *C. sacchariphagus* offspring to develop, and possibly eat parasitised eggs in the same egg batch. This was a major problem in trying to establish a number of *Trichogramma* species on *Eldana saccharina* in South African sugarcane. Egg batches were generally only partially parasitised, allowing *E. saccharina* neonates, which emerged before parasitoids in parasitised egg batches, to eat the unemerged egg parasitoids (Conlong 1997). Field parasitism could also build up fairly rapidly, as often more than one *T. bournieri* emerges from a parasitised *C. sacchariphagus* egg (tab. 4)-another attribute of a good biological control agent (Smith 1996).

The recovery of parasitised eggs (tab. 5) from all the locations where *C. sacchariphagus* lays its eggs (Goebel 1999) shows that this parasitoid has a really good host searching ability, being able to find its host eggs in all positions in the green sugarcane canopy. It also searches in young and old sugarcane with the same tenacity (Conlong *in lit.*). This is probably the most important characteristic to make *T. bournieri* a very successful candidate for an effective augmentation biological control agent (Hassan 1994; Smith 1996).

Because *Trichogramma bournieri* happily accepts *Galleria mellonella* as a factitious host, it can be mass reared in high numbers on this species, as mass rearing technology has been developed for it (King & Hartley 1985), another attribute making an augmentation biological control programme feasible. It is a pity that *Ephestia kuehniella* does not seem to be a good factitious host for *T. bournieri*, as a web search will show that there are a number of commercial insectaries rearing this insect in very high numbers. It is thus easily available for potential Trichogrammatid mass rearing.

Conclusion

The South-east Asian sugarcane borer *Chilo sacchariphagus* is a new introduction into mainland Africa, being identified from two sugar estates in Mozambique. As such it has escaped its indigenous natural enemies. However, it has been colonized by *Trichogramma bournieri*, an African egg parasitoid species whose indigenous host is most likely an indigenous *Chilo* sp. This species has also colonized another exotic maize stem borer, *Chilo partellus*. On *Chilo sacchariphagus* it is a major mortality factor, with attributes detailed in this paper that make it an ideal candidate for an effective augmentation biological control programme.

References

Bonhof M. 2000. *The impact of predators on maize stem borers in coastal Kenya*. PhD Thesis, Wageningen University, Wageningen, the Netherlands, ISBN 90-5808-262-8.

Bournier J.P. 1993. *Trichogramma bournieri* (Pintureau et Babault) Hymenoptera: Chalcididae. Comportement et modifications du cycle biologique à différentes temperatures. *Coton Fibres Tropicales* **48**: 57-62.

Cheng W.Y. 1994. Sugar cane stem borers of Taiwan, p. 97-106 *in:* Carnegie A.J.M., Conlong D.E. (eds.), *Biology, Pest Status and Control Measure Relationships of Sugar Cane Insect Pests*. Proceedings of the second Sugar Cane Entomology Workshop of the International Society of Sugar Cane Technologists. Mount Edgecombe, KwaZulu-Natal, South Africa. ISBN 1-874903-10-7.

Conlong D.E. 1997. Biological control of *Eldana saccharina* Walker in South African sugarcane: Constraints identified from 15 years of research. *Insect Science and its Application* **17**: 69-78.

Conlong D.E., Goebel R. 2002. Biological control of *Chilo sacchariphagus* (Lepidoptera: Crambidae) in Moçambique: the first steps. *Proceedings of the South African Sugar Technologists Association* **76**: 310-320.

Cugala D., Overholt W.A., Santos L., Giga D. 2001.Release of *Cotesia flavipes* Cameron for biological control of cereal stemborers in two ecological zones in Mozambique. *Insect Science and its Application* **21** (3): 303-310.

Goebel F.R. 1999. *Caractéristiques biotiques du foreur de la canne à sucre* Chilo sacchariphagus *(Bojer, 1856) (Lepidoptera: Pyralidae) à l'île de la Réunion. Facteurs de régulation de ses populations et conséquences pour la lutte contre ce ravageur.* PhD thesis, University Paul Sabatier, Toulouse, France, 229 p.

Goebel R., Tabone E., Rochat J., Fernandez E. 2001. Biological control of the sugarcane stem borer *Chilo sacchariphagus* (Lepidoptera: Pyralidae) in Réunion Island: Current and future studies on the use of *Trichogramma* spp. *Proceedings of the South African Sugarcane Technologists Association* **75**: 171-174.

Gonçalves L. 1970. A broco milho *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) em Moçambique. Contribuicao para o sen estudo. *Agronomia Moçambique* **4**: 239-426.

Haile A.T., Hassan S.A., Sithanantham S., Ogol C.K.P.O., Baumgärtner J. 2002. Comparative life table analysis of *Trichogramma bournieri* Pintureau and Babault and *Trichogramma* sp. nr. *mwanzai* Schulten and Feijen (Hym., Trichogrammatidae) from Kenya. *Journal of Applied Entomology* **126**: 287-292.

Hassan S.A. 1994. Strategies to select *Trichogramma* species for use in biological control, p. 55-73 *in:* Wajnberg E., Hassan S. A. (ed.), *Biological Control with Egg Parasitoids.* CAB International, Oxon, UK.

King E.G., Hartley G.G. 1985. *Galleria mellonella*, p. 301-311 *in:* Singh, P., Moore R. F. (eds.), *Handbook of Insect Rearing.* Vol. II, Elsevier, Amsterdam.

Kuniata L.S. 1994. Pest status, biology and effective control measures of sugar cane stalk borers in the Australian, Indonesian and Pacific Island sugar cane growing regions, p 83-96 *in:* Carnegie A.J.M., Conlong D.E. (eds.), *Biology, Pest Status and Control Measure Relationships of Sugar Cane Insect Pests.* Proceedings of the second Sugar Cane Entomology Workshop of the International Society of Sugar Cane Technologists. Mount Edgecombe, KwaZulu-Natal, South Africa, ISBN 1-874903-10-7.

Overholt W.A., Ngi-Song A.J., Kimani S.W., Mbapila J., Lammers P., Kioko E. 1994. Ecological considerations of the introduction of *Cotesia flavipes* for biological control of *Chilo partellus* in Africa. *Biocontrol News and Information* **15**:19N-24N.

Smith S.M. 1996. Biological Control with *Trichogramma*. Advances, Successes and Potential of their Use. *Annual Review of Entomology* **41**: 375-406.

Soula B., Goebel F.R., Caplong P., Karimjee H., Tibere R., Tabone E. 2003. *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) as a biological control agent of *Chilo sacchariphagus* (Lepidoptera: Crambidae) in Réunion Island: Initial field trials. *Proceedings of the South African Sugarcane Technologists. Association* **77**: 278-283.

Tams W.H.T., 1932. New species of African Hetorocera. *Entomologist* **65**: 1241-1249.

Van Rensburg G.D.J., Drinkwater T.W., Carnegie A.J.M., Eulitz E.G., Rust D.J. 1989. Stem Borers, p. 64-73 *in:* Myberg A.C. (ed.), *Crop Pests in Southern Africa.* 4, Bulletin 416, Plant Protection Research Institute, Department of Agriculture and Water Supply, Pretoria.

Way M.J., Turner P.E.T. 1999. The spotted sugarcane borer, *Chilo sacchariphagus* (Lepidoptera: Pyralidae: Crambinae), in Mozambique. *Proceedings of the South African Sugarcane Technologists Association* **73**: 112-113.

Williams J.R. 1983. The sugarcane stem borer (*Chilo sacchariphagus*) in Mauritius. *Revue Agricole Sucrière de l'Ile Maurice* **62**: 5-23.

Factors affecting the bionomics of the eastern African egg parasitoid *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae)

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Abstract. The trichogrammatid *Trichogramma bournieri* Pintureau & Babault is a polyphagous parasitoid of eggs of several cereal stemborer species in eastern Africa. The effects of host species, host age and duration of host deprivation on the performance of the parasitoid were studied in the laboratory. Host acceptance and suitability were tested using five stemborer species. The noctuids *Sesamia calamistis* Hampson, *Sesamia nonagrioides* (Lefebvre), *Busseola fusca* (Fuller) the crambid *Chilo partellus* (Swinhoe) and the pyralid *Eldana saccharina* Walker were successfully parasitized by *T. bournieri*. Parasitism, number of progeny and developmental time varied significantly with host species. The eggs of *S. calamistis* and *B. fusca* were the most suitable, whereas those of *E. saccharina* were the least suitable. While parasitism and number of progeny tended to decrease with age of hosts, there were no significant differences in sex ratio. Longevity of the parasitoid increased with increase in deprivation of hosts from 0 to 12 days. Average lifetime fecundity per female decreased, indicating resorption of eggs.

Résumé. Les facteurs affectant les performances biologiques du parasitoïde d'œufs d'Afrique de l'Est, *Trichogramma bournieri* Pintureau & Babault (Hymenoptera : Trichogrammatidae). Le trichogramme *Trichogramma bournieri* Pintureau & Babault est un parasitoïde polyphage des œufs de plusieurs foreurs de tiges rencontrés en Afrique de l'Est. Les effets de l'espèce et l'âge de l'hôte ainsi que la durée de privation d'hôtes sur sa performance biologique ont été étudiés au laboratoire. Leur développement a été testé sur cinq foreurs de tiges. Les noctuelles *Sesamia calamistis* Hampson, *Sesamia nonagrioides* (Lefebvre), *Busseola fusca* (Fuller) le crambide *Chilo partellus* (Swinhoe) et le pyralide *Eldana saccharina* Walker ont été parasité avec succès par *T. bournieri*. Le parasitisme, le nombre de descendants et la durée de développement ont varié significativement en fonction de l'espèce hôte. Les œufs de *S. calamistis* et *B. fusca* ont le plus faciliter l'émergence des descendants du parasitoïde contrairement à ceux d'*E. saccharina*. Le parasitisme et la descendance ont décrû avec les âges croissants des hôtes, tandis qu'aucune différence significative ne fut trouvée au niveau du sex-ratio. La longévité du parasitoïde s'est accrue avec la durée de privation d'hôtes de 0 à 12 jours alors que la fécondité totale moyenne par femelle a décrû, indiquant la résorption des œufs.

Keywords: *Trichogramma bournieri*, East African stemborers, host suitability, host age, host deprivation.

The most serious pests of cereals across Africa are lepidopteran stemborers belonging to the families Noctuidae, Crambidae and Pyralidae (Polaszek 1998). In East and Southern Africa, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) are the most important species while *Sesamia calamistis* Hampson (Noctuidae), *Chiloorichalcociliellus*(Strand) (Lepidoptera:Crambidae), *Eldana saccharina* Walker (Lepidoptera: Pyralidae) and *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae) are occasional pests (Songa *et al.* 2001). By contrast, in West Africa, *S. calamistis*, *S. nonagrioides* and *E. saccharina* are the most important pests (Buadu

et al. 2003). In Cameroon, Central Africa, *B. fusca* is predominant in all ecozones (Ndemah *et al.* 2003). With the exception of *C. partellus*, which is native to Asia, all other stemborer species are indigenous to Africa.

In the early 1990s, the International Centre of Insect Physiology and Ecology (ICIPE), launched a classical biological control (BC) programme targeting the larval and pupal stages of *C. partellus* (Omwega *et al.* 1995), which since its accidental introduction, has become the most important pest species in lowland areas of eastern Africa (Zhou *et al.* 2000). The "redistribution" approach through exchange of natural enemy species or their strains in different regions of Africa has been proposed as a strategy for controlling indigenous stemborer species (Schulthess *et al.* 1997). For example *Telenomus isis* (Polaszek) (Hymenoptera:

Scelionidae), a parasitoid of eggs of noctuid stemborers (Chabi-Olaye *et al.* 2001), was recently imported from Benin into the quarantine facilities of ICIPE, to be tested against *B. fusca* in East Africa. Egg parasitoids are an important source of mortality because the host is killed before it damages the crop (Temerak 1981). The scanty and mostly anecdotal information from East Africa shows that egg parasitism is low and does not play an important role in the population dynamics of stemborers (Mathez 1972; Okoth 2005). By contrast, in western Africa, *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) and *T. isis* induce up to 100% mortality of eggs of *S. calamistis* and *B. fusca* in the field (Bosque-Pérez *et al.* 1994; Sétamou & Schulthess 1995; Schulthess *et al.* 1997; Schulthess *et al.* 2001; Ndemah *et al.* 2003).

In biological control, competition between species of introduced natural enemies or between introduced and native natural enemies of a pest has been used to explain why some natural enemies fail either to establish or to control the pest (Ehler & Hall 1982; Jalali *et al.* 1988). Thus in a first step, the host range and biotic potential of indigenous parasitoid species should be evaluated before releasing an exotic natural enemy.

In eastern Africa, *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae) is found parasitizing stemborer eggs as well as the African bollworm *Helicoverpa armigera* Hübner (Noctuidae) and the Diamondback Moth *Plutella xylostella* Linnaeus (Yponomentidae) (Haile *et al.* 2002a). Haile *et al.* (2002b) provided some information on the biology and temperature requirements of *T. bournieri* on bollworm and diamondback moth eggs as hosts. No information is available on its biology on eggs of East Africa stemborer species. The present study was completed to provide information on the bionomics of *T. bournieri* as affected by host species, age of host eggs and host deprivation.

Materials and methods

Rearing of hosts and parasitoids

The insect species used were reared at the Animal Rearing and Quarantine Unit (ARQU) of ICIPE in Nairobi. The stemborer species included *C. partellus*, *B. fusca*, *S. calamistis* and *E. saccharina*, together with *S. nonagrioides* collected from wild grasses from Eastern and Western Kenya. *Chilo partellus* was reared on diet developed by Ochieng *et al.* (1985) while the other borer species were reared according to the method described by Onyango & Ochieng-Odero (1994). A colony of *T. bournieri* was established from *B. fusca* eggs that were collected from Mbita (Kenya) and reared following the protocol developed by Chabi-Olaye *et al.* (1997) for rearing of *T. busseolae*.

Host species acceptance

Each stemborer species was allowed to oviposit on three weeks old potted maize plants. Each egg batch was mapped and individual egg numbered. Mated naïve (female without any oviposition experience) *T. bournieri* females which were less than one day old (12 to 16 hours) were used in this experiment. Five couples of each stemborer species were released in each oviposition cage (50 x 15 cm) with two potted maize plants. A piece of cotton wool soaked in water was placed in a small cup as food source for the adults. The females were individually offered one fresh egg batch of about 50 eggs collected from the maize plants. Acceptance was observed under a binocular microscope and was considered as the ability of *T. bournieri* to parasitize the eggs of the five stemborer species tested. After one week, parasitized *T. bournieri* eggs turned dark. Fifteen replications were done for each borer species. The experiment was discontinued if the female did not attack the egg batch after 20 minutes of exposure. If the parasitoid attacked the eggs, the experiment was terminated as soon as the parasitoid left the egg batch and did not come back to the batch after 20 minutes. The females were then removed and eggs incubated at 25 ± 1 °C and 70 ± 5% relative humidity and 12L:12D photoperiod.

Suitability of host age

Only eggs of stemborer species parasitized in the host species acceptance experiment (above) were used. Fifty eggs of each age category (i.e., 1, 2, 3 or 4-days old) were exposed to a naïve mated *T. bournieri* less than one-day-old in a glass vial (28 × 23 mm). For trichogrammatid egg parasitoids, the males emerge first and wait for the females upon which mating takes place (van Djiken & Waage 1987). Virgin males and females were kept together for two hours to ensure that the females were mated. Each treatment (host age) was replicated 15 times in a completely randomized design. After six hours of exposure at 25 ± 1 °C and 70 ± 5% RH, female parasitoids were removed from the vials and exposed eggs incubated at 25 ± 1 °C and 70 ± 5% RH and 12L:12D photoperiod. Data on percentage parasitism, number of progeny, F1 sex ratio (as the proportion of females), and preimaginal developmental time were collected. After two weeks, stemborer eggs from which neither parasitoids nor host larvae emerged were dissected to determine if there were dead parasitoids.

Effect of host deprivation on the reproductive potential of *Trichogramma bournieri*

Less than one day old mated adult parasitoids were fed on 20:80% honey-water solution and left without hosts for 0, 2, 4, 6, 8, 10, and 12 days. From each of these groups, 15 females were isolated individually in glass vials (28 × 23 mm) and 100 eggs provided daily until death of the female. The eggs were transferred to a new vial on a daily basis and incubated in the laboratory at 25 ± 1 °C and 70 ± 5% RH and 12L:12D photoperiod. Female longevity, fecundity, number of progeny and F1 sex ratio (as proportion of females) were determined.

Statistical Analysis

Data on parasitism, development time (in days), total progeny per female, progeny per one host egg and sex-ratio on the different host species were subjected to analysis of variance (ANOVA), using the general linear model (GLM) procedure

of SAS for PC (SAS institute, 1997). Percentage parasitism, sex ratio, total progeny per female and oviposition duration means was separated using Student-Newman-Keuls (SNK) test where ANOVA was significant.

Life table statistics were calculated according to Hulting *et al.* (1990), using the jackknife program. The pre-imaginal survivorship was calculated by dividing the number of individuals alive until adult eclosion by the number of eggs laid by each cohort. This was determined through dissection of the host egg. Difference in intrinsic rate of increase (r_m) values among populations were calculated following the protocol by Dixon (1987) and compared with Newman-Keuls sequential tests (Sokal & Rohlf 1995) based on jackknife estimates of variance for r_m values (Meyer *et al.* 1986). For any difference between two r_m from the sequence to be significant at the α level, the difference must be equal to or greater than

$$LSR = Q_{\alpha[KV]}\sqrt{S^2 av}\sqrt{\frac{n_i + n_j}{2n_i n_j}} \quad [1]$$

Where K is the number of r_m values in the set whose range is tested, and V is the degrees of freedom. The n_i and n_j are the sample sizes of the r_m values, and $Q_{\alpha[K,V]}$ is a value from the table of the Studentized range. S^2_{av} is the weighted average variance of r_m and it is calculated as

$$S^2 av = \frac{\sum^a (n_i - n_j)S^2 i}{\sum^a (n_i - 1)} \quad [2]$$

Where α equals the number of r_m values to be tested, the sample size of the ith r_m is n_i, and S^2_i is the jackknife estimate of the variance for the ith r_m.

Results

Acceptance and suitability of host species and host age

Trichogramma bournieri probed and oviposited in eggs of the all tested stemborer species (*B. fusca, S. calamistis, S. nonagrioides, C. partellus* and *E. saccharina*), but oviposition varied between 46% for *E. saccharina* and 90% for *B. fusca* eggs (tab. 1).

There were significant differences in the number of

Table 1. Acceptability of eggs of several stemborer species to female *Trichogramma bournieri* under laboratory conditions.

Host species	Acceptance	Females ovipositing (%)
B. fusca	+	90.4
S. calamistis	+	81.4
S. nonagrioides	+	71.0
C. partellus	+	47.7
E. saccharina	+	46.0

+, the parasitoid probed and oviposited in host egg.

progeny, parasitism and developmental time among host species (tab. 2). Parasitism and number of progeny tended to decrease with host age. In one-day old eggs, highest parasitism was recorded in *C. partellus*, while lowest was recorded in *E. saccharina* eggs. Parasitism was lowest on *E. saccharina* 2–4 day old eggs and remained similar for the other species.

Number of progeny was lowest on 1–3 day old *E. saccharina* eggs but similar on the remaining species. On 4-day old eggs, both *B. fusca* and *S. calamistis* recorded the highest number of progeny while the least number was observed for *E. saccharina* eggs (tab.2).

Across host age, developmental time was shortest for parasitoids emerging from *B. fusca* eggs but there were no clear trends for the other species. The results show that *T. bournieri* laid more than one egg per host. At age 1, *S. nonagrioides* attended the highest number of eggs laid per host followed by *E. saccharina* and *B. fusca*. The least was observed on *C. partellus* ($F_{4,69} = 7.75$; $p = <.0001$). For all species and across host age, the proportion of females produced did not differ significantly except for *S. calamistis* where the sex ratio decreased as the host age increased ($F_{3,54} = 3.41$; $p = 0.0240$).

Effect of host deprivation on the reproductive potential of *Trichogramma bournieri*

Parasitism, number of progeny and ovipositional period tended to decrease while adult female longevity increased with the increasing days of host deprivation (tab. 3). The effects on sex ratio were minimal except on *S. calamistis* ($F_{6,98} = 2.92$; $p = 0.01$) and *S. nonagrioides* eggs ($F_{6,95} = 2.79$; $p = 0.01$) but there were no clear trends (tab. 3). *Eldana saccharina* eggs were the least suitable host species as indicated by the low parasitism and the number of progeny produced.

Figure 1 shows the cumulative number of progeny of *T. bournieri* in relation to duration of the duration of host deprivation for different host species. The highest proportion of progeny was produced on the first three days in all the treatments and for the all hosts tested (fig. 1). Cumulative numbers of offspring thus increased in a curvilinear manner reaching a plateau as the female grew older. A visual comparison revealed similar rates of progeny within the linear range for all the treatments, but the maxima tended to decrease with days of host deprivation.

Life table parameters

The intrinsic rate of increase (r_m) and net reproductive rate (R_o) were highest on *B. fusca*, then *S. calamistis, S. nonagrioides, C. partellus* and *E. saccharina* eggs in that order (Tab.4).

Doubling time was two times higher for *T. bournieri* reared on *B. fusca* than *C. partellus*. Host deprivation of 0 to 7 days did not affect r_m and R_o but from day 8 onwards, the values decreased (tab. 4).

Discussion

Trichogramma bournieri accepted and successfully developed in a broad range of hosts belonging to Noctuidae, Crambidae and Pyralidae families, though
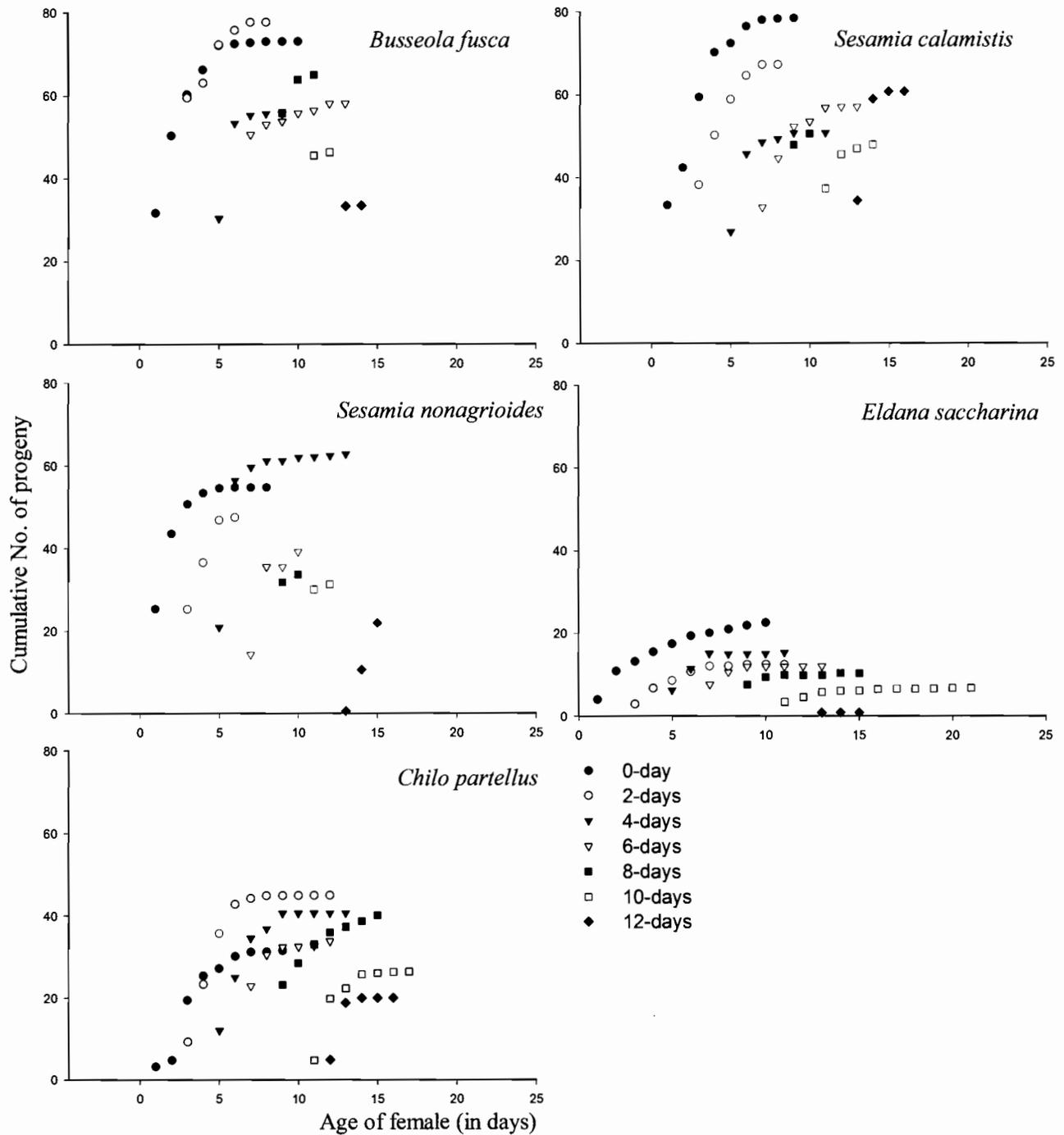

Figure 1
Cumulative number of *Trichogramma bournieri* progeny obtained from different hosts after 0 to 12 days of host deprivation.

Table 2. Acceptance and suitability of eggs at four ages of five East African cereal stemborer species to *Trichogramma bournieri*.

Age (days)	B. fusca	S. calamistis	S. nonagrioides	C. partellus	E. saccharina
			% parasitism		
1	29.8 ± 1.43aA	25.4 ± 1.18aA	17.2 ± 1.83aB	31.3 ± 3.47aA	13.8 ± 2.05aC
2	23.6 ± 1.78bAB	27.8 ± 1.04aA	16.9 ± 0.95aB	26.4 ± 2.06aA	9.4 ± 1.90bC
3	18.8 ± 0.89cA	17.4 ± 1.56bA	19.0 ± 1.07aA	18.6 ± 2.94bA	2.2 ± 0.48cB
4	14.8 ± 0.94dA	17.8 ± 1.71bA	12.0 ± 1.48bA	13.3 ± 1.65cA	2.2 ± 1.65cB
			Number of progeny		
1	51.4 ± 2.99aA	46.5 ± 3.93aA	39.5 ± 4.52aA	38.9 ± 5.17aA	24.8 ± 2.73aB
2	36.6 ± 2.81bA	37.9 ± 2.89abA	28.2 ± 2.69aA	40.9 ± 4.11aA	17.4 ± 3.28bB
3	35.1 ± 2.07bA	30.2 ± 3.45bcA	31.2 ± 2.68aA	25.7 ± 5.19bA	3.8 ± 0.73cB
4	26.4 ± 2.12cA	26.4 ± 3.10cA	17.4 ± 3.04bB	19.4 ± 2.88bB	3.5 ± 0.91cC
			Number of progeny from one host egg		
1	1.73 ± 0.08A	1.84 ± 0.15A	2.33 ± 0.11aA	1.26 ± 0.12B	1.99 ± 0.16A
2	1.58 ± 0.10AB	1.38 ± 0.11B	1.66 ± 0.13bAB	1.56 ± 0.10AB	1.98 ± 0.16A
3	1.87 ± 0.08	1.56 ± 0.20	1.64 ± 0.10b	1.32 ± 0.17	1.84 ± 0.25
4	1.83 ± 0.16	1.58 ± 0.19	1.52 ± 0.17b	1.47 ± 0.16	1.67 ± 0.25
			Development time		
1	8.4 ± 0.27aC	11.0 ± 0.09aB	12.7 ± 0.19aA	11.2 ± 0.85A	10.6 ± 0.19B
2	9.7 ± 0.15aD	10.5 ± 0.19bC	11.4 ± 0.13bB	12.1 ± 0.19A	10.9 ± 0.26C
3	11.3 ± 0.34b	10.3 ± 0.21bc	11.0 ± 0.06c	11.1 ± 0.48	11.2 ± 0.34
4	9.4 ± 2.21aD	10.0 ± 0.00cBC	11.0 ± 0.06cAB	12.4 ± 0.46A	10.4 ± 0.21DC
			Sex ratio		
1	0.87 ± 0.03	0.85 ± 0.02a	0.86 ± 0.02	0.84 ± 0.02	0.75 ± 0.04
2	0.80 ± 0.01	0.83 ± 0.02a	0.78 ± 0.03	0.85 ± 0.03	0.69 ± 0.07
3	0.77 ± 0.06	0.79 ± 0.03ab	0.73 ± 0.03	0.73 ± 0.03	0.77 ± 0.06
4	0.87 ± 0.01	0.72 ± 0.03b	0.71 ± 0.06	0.74 ± 0.06	0.79 ± 0.07

Means within column followed by different lower case letters and means within rows followed by different capital case letters are significantly different (SNK test; $P < 0.05$).

parasitism and the number of progeny varied greatly among host species. *Trichogramma* egg parasitoids are generally polyphagous (Fulmek 1955; Hirai 1988; Kot 1964), with lepidopterans being the main hosts (Thomson & Stinner 1989). Differences in parasitism among species observed in this study may be attributed to the physical stress of the host, which affected the oocyte, resulting in increased permeability of the egg (Waage 1986). Increased mortality of immature *Trichogramma* reported by Calvin *et al.* (1984) and Lund (1934) was attributed to decreasing humidity associated to hardening of eggshells. The relationship between eggshell structure and resistance to desiccation has been studied for several Lepidoptera species (Pak *et al.* 1990). The observed variation in parasitism and the number of progeny between host species might also be attributed to differences in the egg size. *Busseola fusca* eggs are larger than those of *Sesamia* spp. and *E. saccharina* while the latter are larger than those of *C. partellus* (Bruce *in lit.*). Also, Studies by Honda & Luck (2000) indicate that embryological development among hosts varies between species, which might affect

acceptability and suitability of a host. In the present study, *E. saccharina* was clearly the poorest host with only 15% of the eggs being successfully parasitized by *T. bournieri*. This is supported by Conlong & Goebel (2006), who found that *E. saccharina* egg batches were in general not parasitised, and were thus classed as not suitable as a host for *T. bournieri*.

Parasitism and progeny produced in the tested stemborers decreased with age across the host species. The decreasing suitability of older eggs is common among egg parasitoids (Safavi 1968; Fedde 1977; Chabi-Olaye *et al.* 1997; 2001; 2004). This is probably due to the hardening of chorion, which makes it difficult for the parasitoid to probe the egg (Safavi 1968). Similar observations were reported by Sivapragasam & Ahmad (1986), who indicated that the attractiveness of eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) to *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae) decreased with increase in age. Generally, younger host eggs are parasitized more frequently than older ones (Pak 1986; Hints & Andow 1990; Reznik & Umarova 1990; Ruberson & Kring

Table 3. Effect of host egg deprivation on mean longevity, oviposition period in days (d), % parasitism, total progeny, and F1 sex ratio of *Trichogramma bournieri*.

Age (days)	Species				
	B. fusca	*S. calamistis*	*S. nonagrioides*	*C. partellus*	*E. saccharina*
	% parasitism				
0	41.3 ± 2.96a	51.4 ± 3.14a	24.5 ± 2.11b	32.0 ± 2.62b	15.6 ± 2.00c
2	41.7 ± 2.72a	39.0 ± 2.56a	25.1 ± 1.96a	39.0 ± 5.28a	9.4 ± 1.65b
4	34.6 ± 2.70a	29.8 ± 2.42*ab	22.0 ± 2.10b	38.5 ± 3.97a	10.4 ± 2.44c
6	29.8 ± 1.71a	31.4 ± 3.43*a	18.4 ± 2.01b	34.6 ± 2.50a	9.3 ± 1.47c
8	31.1 ± 3.37a	26.4 ± 2.45*a	12.4 ± 1.09*b	35.0 ± 4.60a	8.0 ± 1.05b
10	22.8 ± 2.24*a	20.6 ± 2.17*a	12.6 ± 0.89*a	26.3 ± 4.07a	5.1 ± 1.05*b
12	14.1 ± 1.83*b	29.2 ± 2.22*a	11.2 ± 1.69*b	19.0 ± 5.00*b	1.0 ± 0.49*c
	Progeny				
0	70.9 ± 4.34a	78.4 ± 6.54a	54.7 ± 5.53a	31.5 ± 2.89b	17.5 ± 2.73c
2	77.8 ± 4.34a	67.2 ± 4.50a	47.4 ± 4.44ab	41.5 ± 8.26b	12.4 ± 2.37c
4	58.2 ± 5.82a	60.8 ± 5.88a	57.0 ± 5.23a	40.1 ± 2.99a	16.3 ± 2.82b
6	56.4 ± 3.60a	57.3 ± 5.10a	39.2 ± 4.65b	33.8 ± 2.53b	12.8 ± 2.44c
8	59.8 ± 8.43a	54.0 ± 5.03*a	32.9 ± 3.19a	33.9 ± 5.69a	11.0 ± 1.94b
10	46.3 ± 4.47a	47.9 ± 4.95*a	31.2 ± 2.17ab	26.4 ± 4.29b	8.4 ± 1.92*c
12	33.4 ± 4.33*a	50.4 ± 2.38*a	25.3 ± 4.06*a	19.9 ± 5.96*b	1.2 ± 0.76*c
	Longevity (days)				
0	4.9 ± 0.49ab	3.2 ± 0.11b	4.0 ± 0.53b	6.7 ± 0.68a	6.2 ± 0.63a
2	5.9 ± 0.43*c	6.4 ± 0.44*bc	10.5 ± 0.13*a	7.4 ± 0.38b	6.8 ± 0.49bc
4	6.1 ± 0.25*b	6.5 ± 0.33*b	8.2 ± 0.40*a	8.4 ± 0.52*a	7.8 ± 0.38*a
6	8.9 ± 0.51*	8.4 ± 0.46*	9.2 ± 0.28*	9.4 ± 0.41*	9.1 ± 0.64*
8	10.0 ± 0.20*b	10.3 ± 0.23*b	9.2 ± 0.11*c	12.0 ± 0.35*a	11.4 ± 0.41*a
10	11.3 ± 0.15*c	11.8 ± 0.25*bc	12.7 ± 0.20*b	13.8 ± 0.50*a	12.6 ± 0.41*b
12	13.5 ± 0.13*b	13.5 ± 0.13*b	13.2 ± 0.11*b	14.6 ± 0.25*a	13.6 ± 0.25*b
	Ovipositional period (days)				
0	4.1 ± 0.38	5.0 ± 0.60	2.8 ± 0.41	3.8 ± 0.56	4.3 ± 0.59
2	3.3 ± 0.44	3.9 ± 0.43	2.3 ± 0.25	3.1 ± 0.41	2.7 ± 0.49*
4	1.5 ± 0.21*b	2.0 ± 0.26*ab	3.2 ± 0.44a	3.2 ± 0.45a	2.0 ± 0.46*ab
6	2.7 ± 0.52*	2.4 ± 0.50*	2.3 ± 0.25	2.4 ± 0.40	1.8 ± 0.29*
8	1.5 ± 0.15*b	1.4 ± 0.13*b	1.2 ± 0.10*b	2.5 ± 0.34a	2.0 ± 0.30*ab
10	1.1 ± 0.12*b	1.6 ± 0.23*ab	1.2 ± 0.10*b	2.0 ± 0.28a	1.6 ± 0.42*ab
12	1.0 ± 0.09*a	1.6 ± 0.19*a	1.2 ± 0.14*a	1.0 ± 0.23*a	0.5 ± 0.27*b
	Sex-ratio				
0	0.82 ± 0.02	0.77 ± 0.02	0.72 ± 0.02	0.78 ± 0.03	0.78 ± 0.04
2	0.81 ± 0.03	0.77 ± 0.03	0.82 ± 0.03	0.74 ± 0.03	0.76 ± 0.05
4	0.84 ± 0.02ab	0.87 ± 0.02*a	0.76 ± 0.03ab	0.72 ± 0.04b	0.72 ± 0.04b
6	0.81 ± 0.02ab	0.85 ± 0.02a	0.66 ± 0.03c	0.78 ± 0.03ab	0.71 ± 0.05bc
8	0.71 ± 0.05	0.83 ± 0.02	0.75 ± 0.04	0.71 ± 0.03	0.74 ± 0.04
10	0.86 ± 0.03	0.78 ± 0.02	0.78 ± 0.04	0.81 ± 0.03	0.72 ± 0.05
12	0.73 ± 0.06	0.76 ± 0.02	0.84 ± 0.02*	0.77 ± 0.09	0.96 ± 0.03

Within column, means followed by * are significantly different from the control (0 days of host deprivation) (Dunnett one-tailed t tests), means within row followed by a different lower case letter are significantly different (Student-Newman-Keuls test; P < 0.05).

1993). Pak (1986) hypothesized that rejection of old eggs could be attributed either to the rotation of host embryo or the sclerotization of the head capsule. Monje *et al.* (1999) showed that response to host age is independent of the egg parasitoid species.

The ability of a parasitoid to accept and develop in a broad range of host ages is an advantage during times of host scarcity, given that suitable host stages (fresh egg) may be difficult to find (Chabi-Olaye *et al.* 1997; 2001; 2004). This is especially crucial in eastern Africa, where the cropping season is interrupted by a prolonged dry season, when major pests such as *B. fusca*

Table 4. Life table statistics of *Trichogramma bournieri* on eggs of 5 different stemborer species from females subjected to different periods of host deprivation.

Age (days)	B. fusca	S. calamistis	S. nonagrioides	C. partellus	E. saccharina
		r_m, Jackknife estimate of the intrinsic rate of increase			
0	0.372 ± 0.009a	0.359 ± 0.017ab	0.278 ± 0.006c	0.320 ± 0.019bc	0.202 ± 0.017d
2	0.354 ± 0.008ab	0.362 ± 0.006a	0.296 ± 0.007c	0.334 ± 0.024bc	0.170 ± 0.019d
4	0.356 ± 0.011a	0.359 ± 0.005a	0.268 ± 0.010b	0.343 ± 0.014ab	0.189 ± 0.015c
6	0.369 ± 0.011a	0.298 ± 0.010*b	0.258 ± 0.011bc	0.279 ± 0.010b	0.202 ± 0.031c
8	0.339 ± 0.011*a	0.287 ± 0.013*b	0.273 ± 0.010b	0.271 ± 0.014*b	0.131 ± 0.017*c
10	0.335 ± 0.014*a	0.288 ± 0.008*b	0.257 ± 0.007*c	0.211 ± 0.015*d	0.098 ± 0.028*e
12	0.257 ± 0.015*a	0.277 ± 0.010*a	0.239 ± 0.017*a	0.238 ± 0.031*a	0.009 ± 0.001*b
		R_o, net reproduction rate			
0	46.5 ± 3.4a	40.5 ± 3.6a	29.85 ± 2.8b	22.0 ± 2.1cd	7.6 ± 1.0d
2	45.4 ± 5.4a	40.5 ± 2.3a	35.71 ± 3.3a	29.4 ± 6.0a	6.6 ± 1.2b
4	38.5 ± 4.0a	36.0 ± 1.4a	29.35 ± 3.1ab	27.3 ± 2.4b	8.4 ± 1.5c
6	38.3 ± 3.9a	31.8 ± 3.4ab	23.44 ± 2.5bc	21.5 ± 1.3c	5.0 ± 1.1d
8	43.5 ± 4.9a	32.8 ± 3.9a	18.65 ± 2.8*b	14.6 ± 3.1b	4.7 ± 0.8*c
10	35.7 ± 2.8*a	29.6 ± 2.9*a	19.66 ± 1.5*b	14.6 ± 4.8b	2.8 ± 0.7*c
12	19.2 ± 3.2*b	29.5 ± 3.6*a	16.68 ± 3.1*b	11.8 ± 2.0*b	0.7 ± 0.4*c
		G, mean generation time (day)			
0	10.3	10.3	12.2	9.6	10.0
2	11.8	12.2	12.0	10.1	11.1
4	10.9	12.0	12.6	9.6	11.2
6	7.6	8.0	12.2	9.9	7.9
8	10.6	7.5	11.5	10.9	11.9
10	7.5	11.7	11.5	11.7	10.6
12	11.5	9.8	10.9	11.2	11.5
		t, doubling time (day)			
0	1.8	1.9	2.5	2.1	3.4
2	2.0	2.5	2.3	2.0	4.0
4	2.0	2.4	2.5	2.0	3.6
6	1.4	1.5	2.9	2.5	3.4
8	1.9	1.3	2.5	2.4	5.3
10	1.4	2.4	2.6	3.2	7.0
12	2.7	1.9	2.7	2.9	7.9
		λ, finite rate of increase for population			
0	1.4	1.4	1.3	1.3	1.2
2	1.4	1.3	1.3	1.4	1.1
4	1.4	1.3	1.3	1.4	1.2
6	1.6	1.5	1.2	1.3	1.2
8	1.4	1.6	1.3	1.3	1.1
10	1.6	1.3	1.2	1.2	1.1
12	1.2	1.4	1.2	1.2	1.0

Within column, means followed by * are significantly different from the control (0 days of host deprivation), means within row followed by a different lower case letter are significantly different (Student-Newman-Keuls sequential test; $P < 0.05$).

and *C. partellus* diapause and non-diapausing hosts such as *Sesamia* spp. are scarce (Zhou *et al.* 2000).

The present work shows that a female of *T. bournieri* is capable of ovipositing soon after emergence and lays most of their eggs within the first 12 hours, confirming pro-ovigenic egg production. This was also reported for *T. busseolae* and *T. isis* (Chabi-Olaye *et al.* 1997; 2001).

The sex ratio of parasitoid progeny emerging from all the host species tested was female biased, which is similar to the results from other *Trichogramma* species (Schmidt 1994; Waage 1986), but sex ratio did not change with host species or age of host. Similar results were found for *T. isis* (Chabi-Olaye *et al.* 2001). Parasitoids regulate sex ratio of its progeny based on the total number of

eggs laid and the clutch size (Yu *et al.* 2003). However, clutch size did not vary in the present study.

The host deprivation study showed that depending on the host species, *T. bournieri* could withhold from ovipositing for up to ten days without any negative effects on fecundity, but adult longevity was prolonged considerably. This corroborates findings by Chabi-Olaye *et al.* (1997; 2001) working on *Telenomus* spp., though the egg retention capacity of these species was much higher than that of *T. bournieri*. In contrast, parasitism by *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) was drastically reduced possibly due to egg resorption when parasitoids were subjected to host deprivation for 4 days (Fleury & Boulétreau 1993). The decreasing total fecundity in the current study might be due to both the decreasing numbers of viable eggs and egg resorption. These findings support the trade-off hypothesis of negative correlation between reproduction and adult survival (Calow 1973; Bell & Koufopanou 1986). According to Bell & Bohm (1975), egg resorption among insects is an adaptive strategy that allows females to conserve their metabolic resources instead of laying eggs under unfavourable conditions (Garcia *et al.* 2001). These results show that female wasps approaching the end of their lives probably optimised their fitness, when equalizing the risks of being egg depleted. According to the "static optimisation model" of host acceptance, older wasps tend to maximize their progeny production by ovipositing a higher number of eggs as soon as suitable hosts are discovered. In this model, parasitoids maximize their rate of gain of a quantity, such as the number of progeny produced (Godfray 1994). This was also demonstrated by the short oviposition period for *T. bournieri* in this study.

The present findings indicate that *T. bournieri* has a higher biotic potential and that the host range is wider than that of *T. isis*. Furthermore, *T. isis* only attacks eggs of noctuids which are concealed between the leaf sheath and the stem (Chabi-Olaye *et al.* 2001), while *T. bournieri*, in addition, also attacks eggs laid on the surface of leaves. Bruce (*in lit.*) showed that parasitism of noctuid eggs by *T. bournieri* is only about 7% in nature, while Haile *et al.* (2000) and Conlong & Goebel (this issue) report that parasitism of crambid eggs vary between 38 and 90%. Thus the niche overlap of the two parasitoid species is small and interspecific competition would be minimal.

The suitability of eggs of *E. saccharina*, the only non-noctuid stemboring pest of maize in West Africa, for *T. bournieri* was shown to be low. Thus, *T. bournieri* cannot be considered a suitable 'redistribution' candidate for control of stemborers in western Africa.

References

Bell G., Koufopanou V. 1986. The cost of the reproduction. *Oxford Surveys in Evolutionary Biology* 3: 83-131.

Bell W., Bohm M. K. 1975. Oosorption in insects. *Biological Review* 50: 373-396.

Bosque-Pérez N.A., Ubeku J.A., Polaszek A. 1994. Survey for parasites of *Sesamia calamistis* (Lep.: Noctuidae) and *Eldana saccharina* (Lep.: Pyralidae) in southwestern Nigeria. *Entomophaga* 39: 670-376.

Buadu E.J., Gounou S., Cardwell K.F., Mochiah B., Botchey M., Darkwa E., Schulthess F. 2003. Distribution and relative importance of insect pests and diseases in Southern Ghana. *African Plant Protection* 8: 3-11.

Calow P. 1973. The relation between fecundity, phenology and longevity: A system approach. *American Naturalist* 107: 559-574.

Calvin D.D., Knapp M.C., Welch S.M., Poston F.L., Elzinga R.J. 1984. Impact of environmental factors on *Trichogramma pretiosum* reared on Southwestern corn borer eggs. *Environmental Entomology* 13: 774-780.

Chabi-Olaye A., Fiaboe M.K., Schulthess F. 2004. Host preference, Suitability and Thermal Requirements of *Lathromeris ovicidia* Risbec (Hymenoptera: Trichogrammatidae) Egg Parasitoid of Cereal Stemborers in Africa. *Biological Control* 30: 617-623.

Chabi-Olaye A., Schulthess F., Poehling H.-M., Borgemeister C. 1997. Factors influencing the developmental rates and reproductive potentials of *Telenomus busseolae* (Gahan) (Hymenoptera: Scelionidae), an Egg Parasitoid of *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Biological Control* 8: 15-21.

Chabi-Olaye A., Schulthess F., Poehling H.-M., Borgemeister C. 2001. Factors Affecting the Biology of *Telenomus isis* (Polaszek) (Hymenoptera: Scelionidae), an Egg Parasitoid of Cereal Stem Borers in West Africa. *Biological Control* 21: 44-54.

Conlong D.E., Goebel F.R. 2006. *Trichogramma bournieri* Pintureau and Babault (Hymenoptera: Trichogrammatidae) and *Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae) in surgarcane in Mozambique – A new association. *Annales de la Société entomologique de France (N.S.)* 42(3-4): 417-422.

Dixon A.F.G. 1987. Parthenogenetic reproduction and the rate of increase in aphids, p. 269-285 *in*: Minks A. K., Harrewijin P. (eds.), *Aphids: Their Biology, Natural Enemies and Control.* Elsevier, Amsterdam.

Ehler L.E., Hall R.W. 1982. Evidence for competitive exclusion of introduced natural enemies in biological control. *Environmental Entomology* 11: 1-4.

Fedde G.F. 1977. A laboratory study of egg parasitism capabilities of *Telenomus alsophilae* Viereck. *Environmental Entomology* 6: 773-776.

Fleury F., Boulétreau M. 1993. Effects of temporary host deprivation on the reproductive potential of *Trichogramma brassicae*. *Entomologia Experimentalis et Applicata* 68: 203-210.

Fulmek L. 1955. Wirtsbereich von *Trichogramma evanescens* Westw und *T. minutum* Ril. Anz. *Für Schädlingskunde* 8: 113-116.

Garcia V.P., Wajnberg E., Oliveira M.L.M, Tavares J. 2001. Is the parasitization capacity of *Trichogramma cordubensis* influenced by the age of the females? *Entomologia Experimentalis et Applicata* 98: 219-224.

Godfray H.C.J. 1994. *Parasitoids: Behavioral and Evolutionary Ecology.* Princeton University Press N. J., 473p.

Haile A.T., Hassan S.A., Ogol C.P.K.O., Baumgärtner J., Sithanantham S., Monje J.C. 2002a. Comparative life table analysis of *Trichogramma bournieri* Pintureau and Babault and *Trichogramma* sp. nr. *mwanzai*

Shulten and Feijen (Hymenoptera: Trichogrammatidae) from Kenya. *Journal of Applied Entomology* 126: 287-292.

Haile A.T., Hassan S.A., Ogol C.P.K.O., Baumgärtner J., Sithanantham S., Monje J.C., Zebitz C.P.W. 2002b. Temperature-dependent development of four egg parasitoid *Trichogramma* species (Hymenoptera: Trichogrammatidae). *Biocontrol Science and Technology* 12: 555-567.

Haile A.T., Sithanantham S., Ogol C.K.P.O., Monje J.C., Zebitz C.P.W. 2000. Survey for native Trichogrammatid species in Kenya. *Proceeding of XXI International Congress of Entomology, August 20-26, 2000 Iguassu, Brazil,* 389p.

Hints J.L., Andow D.A. 1990. Host age and host selection by *Trichogramma nubilale*. *Entomophaga* 35: 141-152.

Hirai K. 1988. The parasitism of *Trichogramma chilonis* T. on eggs of the soybean pod borer *Leguminivora glycinivorella* M. in soybean field of Northern Japan. *Colloques de l'INRA* 43: 479-489.

Honda J.Y., Luck R.F. 2000. Age and Suitability of *Amorbia cuneana* (Lepidoptera : Tortricidae) and *Sabulodes aegrotata* (Lepidoptera: Geometridae) Eggs for *Trichogramma platneri* (Hymenoptera: Trichogrammatidae). *Biological Control* 18: 79-85.

Hulting F.L., Orr D.B., Obrycki J.J. 1990. A computer programme for calculation and statistical comparison of intrinsic rates of increase and associated life-table parameters. *Entomologist* 73: 601-612.

Jalali S.K., Singh S.P., Ballal C.R., Kumar P. 1988. Competitive interaction between *Cotesia Kazak* and *Hyposoter didymator*, exotic parasitoids of *Heliothis armigera*. *Entomologia Experimentalis et Applicata* 46: 221-225.

Kot J. 1964. Experiments in the biology and ecology of species of the genus *Trichogramma* Westw. and their use in plant protection. *Ekologia Polska-Series A* 12: 243-303.

Lund H.O. 1934. Some temperature and humidity relations of two races of *Trichogramma minutum* Riley (Hym. Chalcididae). *Annals of Entomological Society of America* 27: 324-340.

Mathez F.C. 1972. *Chilo partellus* Swinh., *Chilo orichalcociliella* Strand (Lep.: Crambidae) and *Sesamia calamistis* Hmps. (Lep.: Noctuidae) on maize in the Coast province, Kenya. Mitteilungen der Scweizerischen. *Entomologischen Gesellschaft* 45: 267-289.

Meyer J.S., Ingersoll C.G., McDonald L.L., Boyce M.S. 1986. Estimating uncertainty in population growth rates: Jackknife vs. bootstrap techniques. *Ecology* 67: 1156-1166.

Monje J.C., Zebitz C.P.W., Ohnesorge B. 1999. Host and Host Age Preference of *Trichogramma galloi* and *T. pretiosum* (Hymenoptera: Trichogrammatidae) Reared on Different Hosts. *Biological and Microbial Control* 92: 97-103.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Poehling M., Cardwell K.F. 2003. Factors affecting infestations of the stalk borer *Busseola fusca* (Fuller) on maize in the forest Zone of Cameroon with special reference to scelionid egg parasitoids. *Environmental Entomology* 32: 61-70.

Ochieng R.S., Onyango F.O., Bungu M.D.O. 1985. Improvement of techniques for mass culture of *Chilo partellus* (Swinhoe). *Insect Science and its Applications* 6: 425-428.

Okoth E.O.R. 2005. *Studies on species diversity, host preference, host suitability, longevity and fecundity of native egg parasitoids of stemborers in semi-arid Kenya*. MSc. thesis. Kenyatta University, 59p.

Omwega C.O., Kimani S.W, Overholt W.A. 1995. Evidence of the establishment of *Cotesia flavipes* (Hymenoptera: Braconidae) in continental Africa. *Bulletin of Entomological Research* 85: 525-530.

Onyango F.O., Ochieng-Odero J.P.R. 1994. Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis et Applicata* 73: 139-144.

Pak G.A. 1986. Behavioral variations among strains of *Trichogramma* spp. A review of the literature on host-age selection. *Journal of Applied Entomology* 101: 55-64.

Pak G.A., Dalen A., van Kaashoek N., Djikman H., Van-Dalen A. 1990. Host chorion structure influencing host suitability for the egg parasitoid *Trichogramma maidis* Westwood. *Journal of Insect Physiology* 36: 869-875.

Polaszek A. 1998. *African cereal stemborers: economics importance, taxonomy, natural enemies and control.* CAB International, Wallingford, Oxon, United Kingdom, 530p.

Reznik S.Y., Umarova T.Y. 1990. The influence of host's age on the selectivity of parasitism and fecundity of *Trichogramma*. *Entomophaga* 35: 31-37.

Ruberson J.R., Kring T.J. 1993. Parasitism of developing eggs by *Trichogramma pretiosum* (Hymenoptera: Ttichogrammatidae): Host age preference and suitability. *Biological Control* 3: 39-46.

Safavi M. 1968. Etude biologique et écologique des Hyménoptères parasites des oeufs des punaises des céréales. *Entomophaga* 13: 381-495.

SAS Institute 1997. *SAS/STAT Software: changes and enhancements through Release.* 6. 12. Cary, North Carolina.

Schmidt J.M. 1994. Host recognition and acceptance by *Trichogramma*, p. 165-199 *in*: Wajnberg E., Hassan S. A. (eds), *Biological Control with Egg Parasitoids.* CAB International, Oxon, UK.

Schulthess F., Bosque-Pérez N.A., Chabi Olaye A., Gounou S. Ndemah R., Goergen G. 1997. Exchange of natural enemies of Lepidopteran Cereal Stemborers between Africa regions. *Insect Science and its Application* 17: 97-108.

Schulthess F., Chabi-Olaye A., Goergen G. 2001. Seasonal Fluctuations of Noctuid Stemborer Egg Parasitism in Southern Benin with Special Reference to *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Telenomus spp.* (Hymenoptera: Scelionidae) on Maize. *Biocontrol Science and Technology* 11: 745-757.

Sétamou M., Schulthess F. 1995. The influence of egg parasitoids belonging to the *Telenomus busseolae* (Hymenoptera: Scelionidae) species complex on *Sesamia calamistis* (Lepidoptera: Noctuidae) population in maize fields in Southern Benin. *Biocontrol Science and Technology* 5: 69-81.

Sivapragasam A., Ahmad A. 1986. Parasitism by *Trichogramma australicum* Gir. on *Corcyra cephalonica* Stainton eggs of various ages. *MARDI-Research-Bulletin* 12: 205-210.

Sokal R.R., Rolf J.F. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research.* 3rd ed. Freeman, New York.

Songa J.M., Guofa Z., Overholt W.A. 2001. Relationships of stemborer damage and plant physical condition to maize yield in a semi-arid zone of eastern Kenya. *Insect Science and its Application* 21: 243-249.

Temerak S.A. 1981. Qualitative and quantitative survey on the oophagous wasp attacking the pink borer, *Sesamia cretica* Led. (Lep.: Noctuidae) on 3 graminacious crops in Upper Egypt. *Zeitschrift für Angewandte Entomologie* 1: 398-402.

Thomsom M.S., Stinner R.E. 1989. *Trichogramma* spp. (Hymenoptera: Trichogrammatidae): field hosts and multiple parasitism in North Carolina. *Journal of Entomological Science* 24: 232-240.

van Djiken M.J., Waage J.K. 1987. Self- and superparasitism by the egg parasitoid *Trichogramma evanescens*. *Entomologia Experimentalis et Applicata* 43: 183-192.

Waage J.K. 1986. Family planning in parasitoids: adaptative patterns of progeny and sex allocation, p. 63-95 *in*: Waage J. K., Greathead D. J. (eds), *Insect Parasitoids. 13th Symposium of the Royal Entomological Society of London.* Academic Press, London, UK.

Yu S.-H., Ryoo M.I., Na J.H., Choi W.I. 2003. Effect of host density on egg dispersion and the sex ratio of progeny of *Bracon hebetor* (Hymenoptera : Braconidae). *Journal of Stored Products Research* 39: 385-393.

Zhou G., Baumgartner J., Overholt W.A. 2000. Impact of an exotic parasitoid on stemborer (Lepidoptera) populations dynamics in Kenya. *Ecological Applications* 11: 1554-1559.

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Calyx fluid proteins of two *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) biotypes in Kenya: implications to biological control of the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae)

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Abstract. *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) is an indigenous larval endoparasitoid of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in sub-Saharan Africa. In Kenya, reports suggest that *C. sesamiae* occurs as two biotypes. Biotype avirulent to *B. fusca* gets encapsulated by haemocytes in this host and is unable to complete development. Biotype virulent to *B. fusca* is able to overcome immune defences. Factors present in the calyx fluid such as the PolyDNAviruses (PDV), venom and calyx fluid proteins have been implicated in the variation of *C. sesamiae* virulence against *B. fusca*. In the present study, calyx fluid proteins of the two *C. sesamiae* biotypes were compared using 2-D gel electrophoresis. More protein spots were observed in the virulent parasitoid calyx fluid, but some proteins were specifically observed in the avirulent parasitoid calyx fluid while others were observed in both. To study changes in proteins due to parasitism of *B. fusca* larvae by the two strains, SDS-PAGE gel were performed on fat body tissues and the haemolymph at three time points. Differences between the two strains were observed in both the fat body and haemolymph tissues. Parasitism-specific protein bands were detectable in fat body tissues of *B. fusca* larvae parasitized by the two *C. sesamiae* strains. These proteins were absent in unparasitized larvae. Implications for using *C. sesamiae* as a biocontrol agent of *B. fusca* in Africa are discussed.

Résumé. Protéines des fluides du calyx de deux biotypes de *Cotesia sesamiae* (Cameron) (Hymenoptera : Braconidae) au Kenya: implications pour le contrôle biologique du foreur *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae). *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) est un parasitoïde larvaire indigène de *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) en Afrique sub-saharienne. Au Kenya *C. sesamiae* existe sous forme de deux biotypes. Le biotype avirulent pour *B. fusca*, encapsulé par les hémocytes de cet hôte est incapable de s'y développer. Le biotype virulent est capable de contourner ses défenses immunitaires de *B. fusca*. Des facteurs présents dans le fluide du calyx de la guêpe ont été impliqués dans ces variations de virulence. Dans la présente étude, les protéines du calyx de guêpes appartenant aux deux biotypes furent comparées sur gel d'électrophorèse bidimensionnelle. Globalement, plus de spots protéiques furent observés chez la souche virulente, mais certains furent observés spécifiquement chez le biotype virulent, d'autres enfin, chez les deux biotypes du parasitoïde. Afin de comparer la localisation dans la larve et dans le temps des changements protéiques dus aux infestations par les deux biotypes, des gels SDS-PAGE furent réalisés à partir d'extraits de l'hémolymphe et du corps gras prélevés à trois intervalles de temps après l'infestation. Les différences entre les souches furent observées pour les deux tissus. Certaines protéines, spécifiques du parasitisme, absentes des larves non parasitées, furent observées dans le corps gras des larves parasitées par les deux souches de parasitoïde. Les implications pour l'utilisation de *C. sesamiae* comme agent de lutte biologique sont discutées.

Keywords: *Busseola fusca*, *Cotesia sesamiae*, Stem borer, Endoparasitoid, Africa.

Hymenoptera endoparasitoids spend their life cycle inside other insects, generally Lepidoptera hosts. The host immune system can perceive the parasitoid's egg as foreign, and respond by mounting an encapsulation reaction that can lead to the egg's

death (Ratcliffe 1993). A variety of mechanisms have been developed by parasitoids to overcome the defence reactions of their natural hosts. The most well studied mechanism is immune suppression induced by symbiotic viruses known as PolyDNAviruses (PDV) (Asgari & Schmidt 1994a; Hayakawa & Yazaki 1997; Beckage 1998; Drezen *et al.* 2000).

Many hymenopteran parasitoids contain viruses and other components in their ovaries which are co-

injected together with the eggs and specifically interfere with the hosts' internal defence mechanisms (Fleming 1992; Stolz 1992). They also manipulate the hosts' physiology in order to accommodate and favour the developing parasitoid larvae (Vinson 1990). Parasitoid females in several genera of parasitoid wasps from Ichneumonid and Braconid families produce PDVs in the calyx gland of their ovaries that are injected into the host during parasitism and are disrupting to the host immune response. The PDV DNA is also present as integrated proviruses in the parasitoids chromosomes (Savary et al. 1997; Belle et al. 2002). Certain PDV genes are transcribed and translated and exert effects on the lepidopteran host, including disruption of host immune systems and protein synthesis in ways that favour parasitoid survival. The calyx fluid injected into the host along with the eggs also contains ovarian and venom proteins.

It is known that some host insects react quickly to foreign objects that are introduced into their haemolymph (Ratcliffe 1993). The time that is required to express viral genes in the host cells (Theilmann & Summers 1986) and to change the immune status of the host (Stoltz 1986) probably exceeds the time it takes to encapsulate the egg of the parasitoid. Calyx fluid proteins have been known to offer early protection of eggs before PDV expression. In the parasitoid *Cotesia rubecula* (Marshall 1885) (Hymenoptera: Braconidae), early protection of eggs by calyx fluid proteins has been shown in the host *Pieris rapae* L. 1758 (Lepidoptera: Pieridae) (Asgari & Schmidt 1994b). Calyx fluid proteins and egg surface proteins hence could provide passive protection to the parasitoid eggs from encapsulation by the host. Although the mechanisms involved in immune suppression have been studied extensively in many systems, the factors involved in its natural variation remain little studied, especially for PDV carrying wasps.

Cotesia sesamiae (Cameron 1891) (Hymenoptera: Braconidae) is a gregarious koinobiont endoparasitoid that is widespread in Africa (Mohyuddin 1990; Polaszek & Walker 1991) and attacks mid- to late- instar stem borer larvae. This parasitoid attacks several lepidopteran stem borer larvae including *Sesamia calamistis* Hampson 1910 (Noctuidae), *Busseola fusca* (Fuller 1901) (Noctuidae), *Chilo partellus* (Swinhoe 1885) (Crambidae) and *Chilo orichalcociliellus* (Strand 1911) (Crambidae) (Mohyuddin 1971; Polaszek & Walker 1991). Among the complex of stem borers on maize and sorghum in sub-Saharan Africa, only *B. fusca* are able to mount an immune response against *C. sesamiae*. A study by Ngi-Song et al. (1998) showed that *C. sesamiae* from the Kenyan coast does not develop in *B. fusca*, whereas

C. sesamiae from Kitale successfully develops in *B. fusca*. The fact that *C. sesamiae* exists in two biotypes that react differently to *B. fusca* immune reactions raises a few questions about the physiological differences that may exist between the two parasitoid populations.

Studies by Mochiah et al. (2002) showed that eggs of *C. sesamiae* from Kenyan coast that normally do not develop in *B. fusca*, developed when the host was injected with calyx fluid of *C. sesamiae* from Kitale prior to oviposition. This indicates that factors in the calyx fluid are responsible for disarming the immune system of *B. fusca* and that the factors from the two *C. sesamiae* biotypes are physiologically and genetically different. PDV expression has been detected in haemocytes, fat bodies and other tissues in some parasitoid systems as early as four hours post- parasitism (Webb & Luckhart 1994). Calyx fluid proteins and viral proteins play a vital role in the encapsulation response of the host in the presence of a functional PDV (Hayakawa 1994; Asgari & Schmidt 1994b). Venom and ovarian proteins are introduced directly into the haemolymph during parasitization where they may target the haemocytes or other components of the host immune system.

There is renewed interest in the redistribution of *C. sesamiae* as a biological control agent of stem borers in Africa (Schulthess et al. 1997). The location from where *C. sesamiae* would be drawn from during the releases and the strain of the parasitoid to be used need to be known depending on the investment in resistance of the target host species. In order to identify parasitoid or host proteins involved in the variation in *C. sesamiae* virulence, we compared calyx protein migration patterns in the two *C. sesamiae* biotypes as well as in *B. fusca* larvae parasitized by the two biotypes as opposed to unparasitized ones.

Materials and methods

Insects collection and rearing

Insects were collected from farmers' fields in Kitale and Mombasa, Kenya (fig. 1). Plants that exhibited signs of stem borer attack or feeding were randomly picked, dissected and all the stem borer larvae and parasitoid cocoons found in the stems placed individually in glass vials (7.5 cm x 2.5 cm). The larvae were provided with a piece of maize stem or artificial diet (Onyango & Ochieng-Odero 1994). The collected material was transported to the laboratory in ICIPE, Nairobi and the larvae were observed for cocoon formation and parasitoids emergence. Adult *Cotesia* spp. that emerged from cocoons were identified using the shape of male genitalia or the propodia in all-female broods (Kimani-Njogu & Overholt 1997). Upon identification, *Cotesia sesamiae* progeny were allowed to mate under light in a vial. *C. sesamiae* females from Kitale were reared on *B. fusca*, while Mombasa *C. sesamiae* were reared on *S. calamistis* larvae. The stem borers were hosts from which the
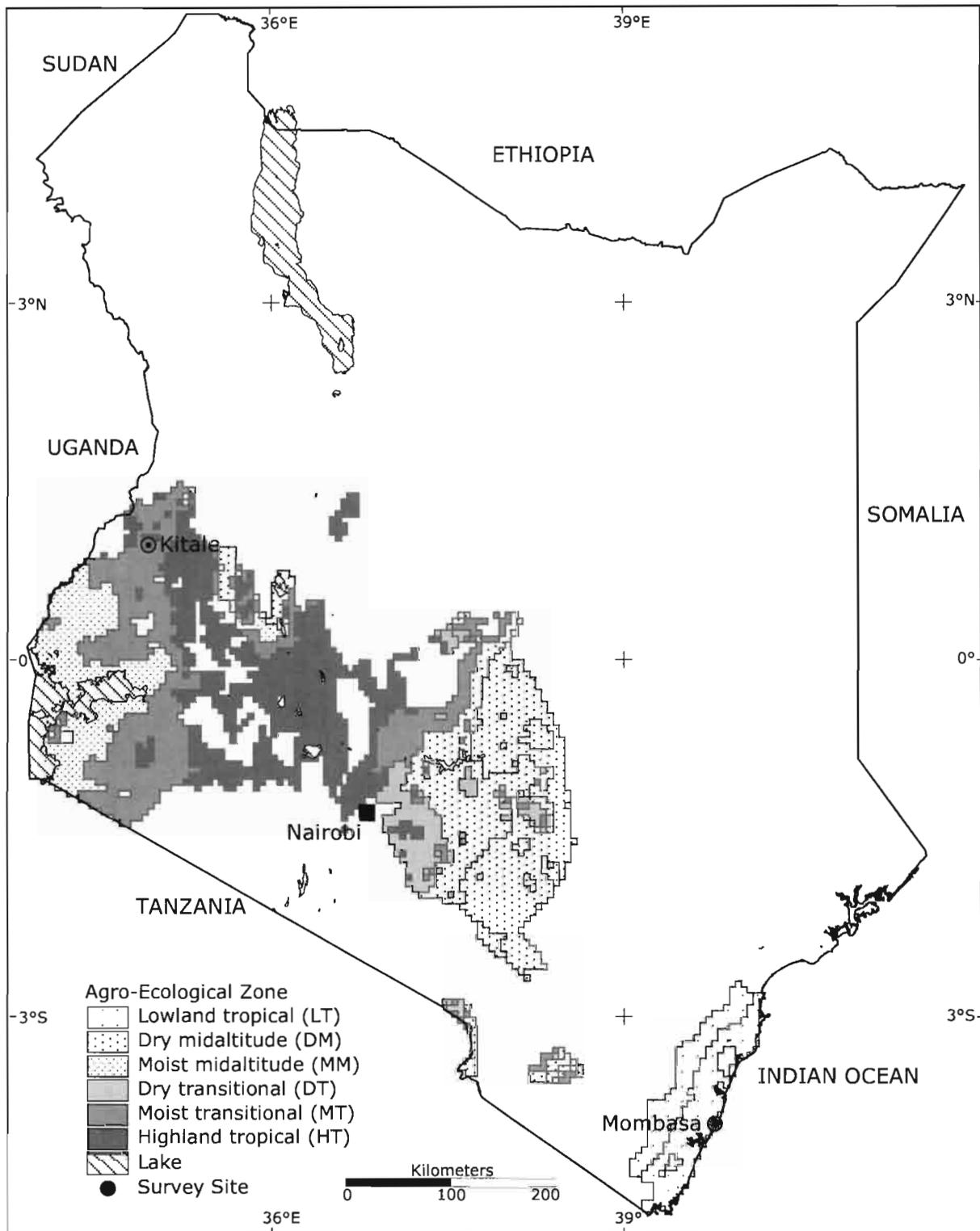
Figure 1
Map of Kenya showing the two geographic locations where *Cotesia sesamiae* and *Busseola fusca* were collected. The coastal Mombasa *C. sesamiae* population was collected from Mtwapa.

parasitoids emerged from. Larvae were placed in artificial diet at 25 ± 1 °C until cocoon formation and later wasp's emergence. Progeny that emerged were used for the bioassays.

Collection of calyx fluid

Mated two- to three- days old *C. sesamiae* females from Kitale and Mombasa were used for the experiment. 50 female wasps were selected from the rearing cages, put in a vial and immobilized on ice prior to dissection. A drop of phosphate buffer saline (PBS pH 7.0) was placed on a Petri dish and dissection carried out on ice blocks. Using sharp dissecting forceps, the intersegmental membranes between the posterior abdominal and the dorsal part of the abdominal segments of the female *C. sesamiae* were teased out. The ovipositor was grasped and pulled free to remove the reproductive system. Upon each single dissection, the ovaries were placed in an Eppendorf tube containing 100 μl protease inhibitor cocktail (Sigma P2714)
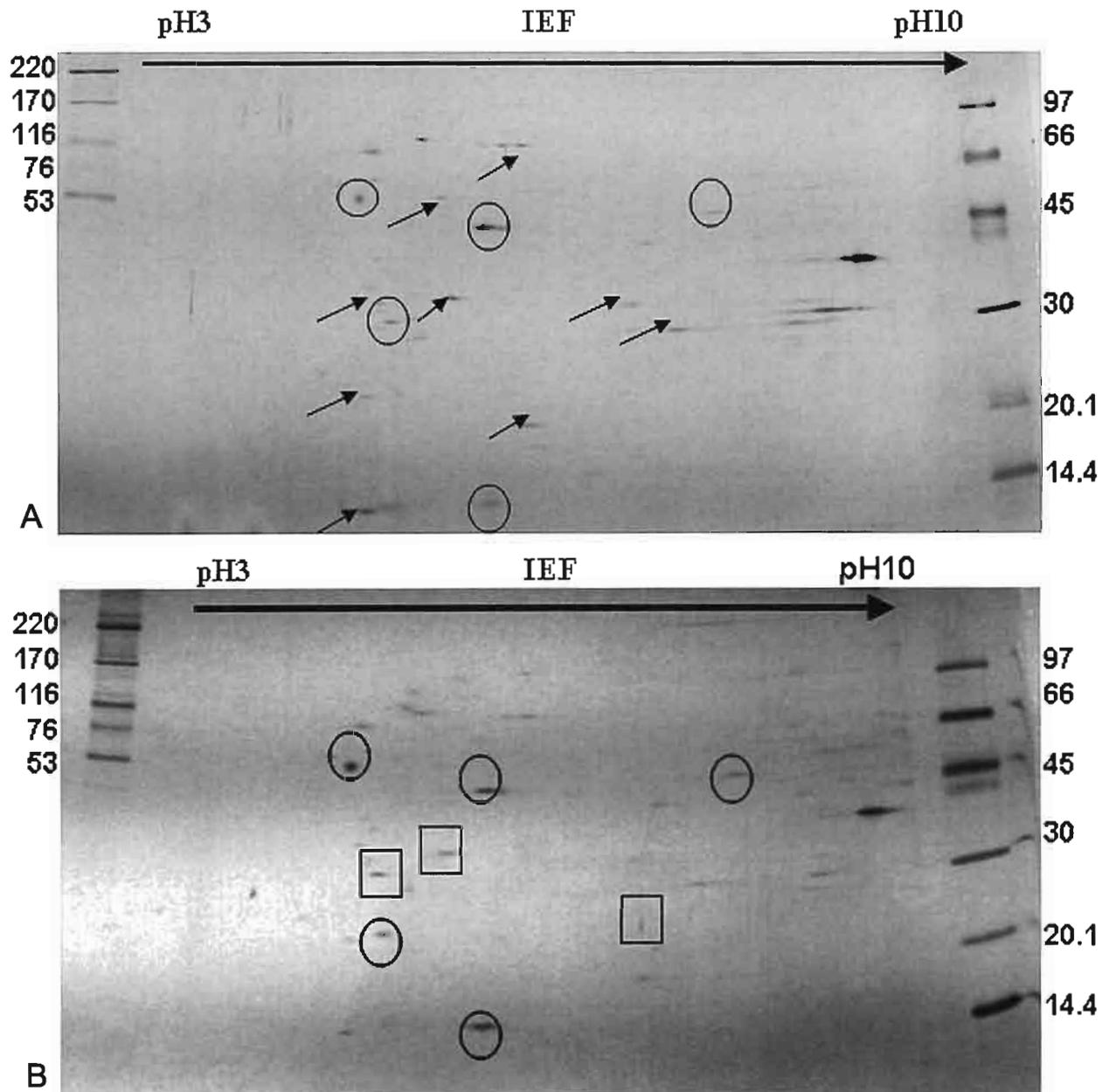
Figure 2
IEF 2-D gel migration of calyx fluid from virulent (A) and avirulent (B) biotypes of *C. sesamiae*. First dimension: pH 3-10; second dimension 4-20 % acrylamide. Open circles: protein spots present in both virulent and avirulent line calyx fluids. Arrows: protein spots unique to the virulent line calyx fluid. Open squares: protein spots unique to the avirulent line calyx fluid. Only the prominent spots were considered.

in PBS. The samples of the ovaries from each location were pooled together and maintained on ice until all dissections were completed. To shear the ovaries and release the calyx fluid, the ovaries were drawn in and out into a 17 G syringe followed by a 23 G syringe for 5 minutes each syringe. The contents were then centrifuged for 4 minutes at 3000 g at 4 °C. The supernatant was collected and transferred into a clean-labelled centrifuge tube and placed on ice.

2-D gel electrophoresis on calyx fluid proteins of *Cotesia sesamiae* Kitale and Mombasa strains

2D-PAGE was performed as described by O'Farrell (1975). For each sample, 60 µl calyx fluid supernatant was added to 60 µl of IEF sample buffer and 60 mg of urea. Urea was mixed by gently tapping the sample until all the particles dissolved. For the first dimension (isoelectric focusing, IEF) we used gradient gels covering the range pH3 to pH10 (ampholines, Millipore Inc). The IEF gels were run at 160 V for 16 hrs and then 320 Volts for 1 hr. We used 4-20% acrylamide gels for the second dimension. The 2-D gels were silver stained as described by Morrissey (1981).

Protein profiles for haemolymph and fat bodies of larvae parasitized at different time points

Cotesia sesamiae were allowed to oviposit on *B. fusca* and *S. calamistis* larvae using the hand-sting method (Overholt *et al.* 1994). Larvae were placed in artificial diet until dissections 6, 12 or 24 hours post oviposition. The larvae were washed with 70% ethanol and rinsed in distilled water before collection of fat bodies and haemolymph. The abdominal proleg was snipped to release haemolymph into an Eppendorf tube containing 100 µl protease inhibitor. Care was taken not to rupture the gut and any samples that were contaminated were discarded. Fat bodies were thereafter dissected and other tissues carefully removed and discarded. The fat body tissues were washed in PBS five times to remove haemolymph residues and thereafter placed in 100 µl protease inhibitor.

Proteins in both fat body and haemolymph samples were purified under denaturing conditions using Urea, Tris-Cl and Sodium monophosphate. The samples were prepared for Sodium Dodecyl Sulphate –Polyacrylamide Gel Electrophoresis (SDS-PAGE) by boiling 100 µl of sample with equal amounts of disruption mix (Glycerol, SDS, ß mercaptoethanol and Tris-HCL and bromophenol) blue for five minutes. Fat body
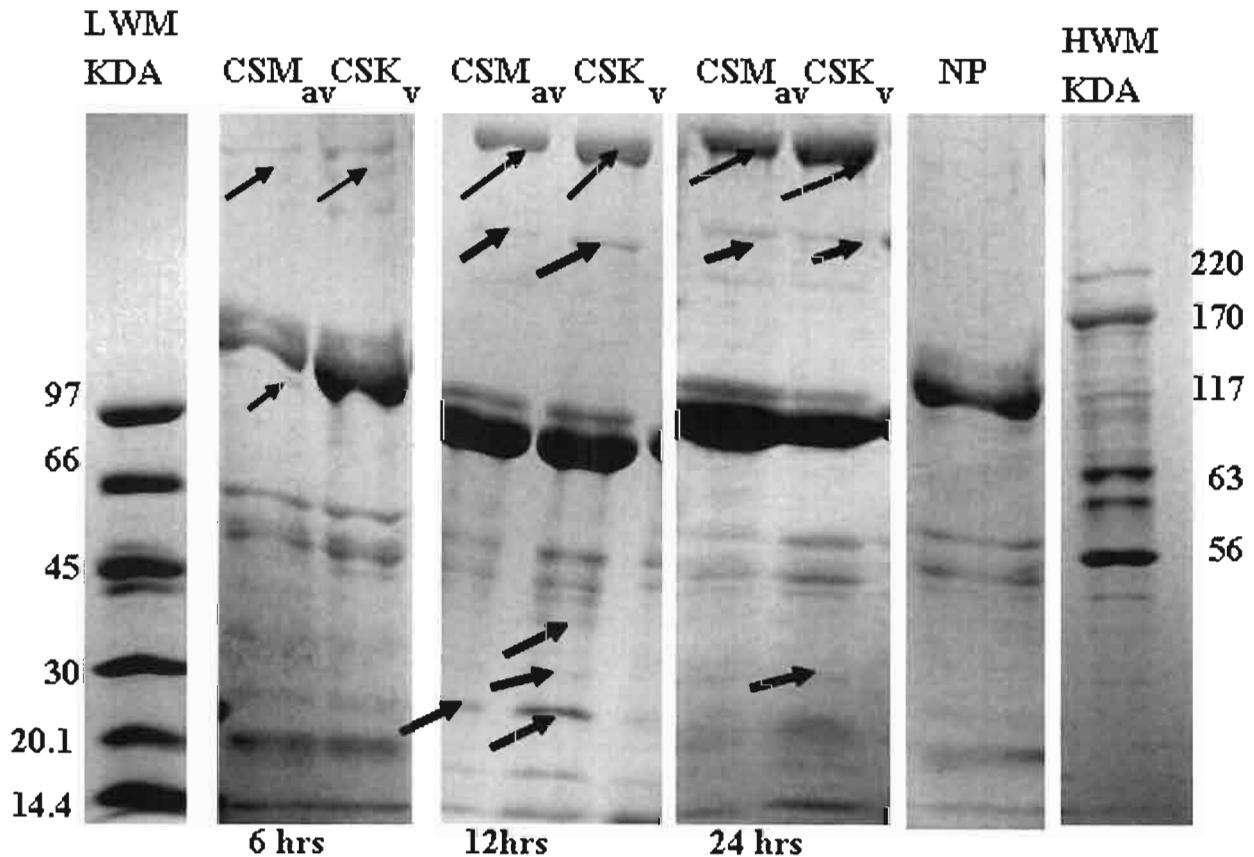

Figure 3
SDS-PAGE migration (4-20%) of proteins from *Busseola fusca* tissues dissected from larvae parasitized by virulent and avirulent lines of *Cotesia sesamiae* at different time points post-infestation. The bands were compared with haemolymph from non-parasitized larvae at 12 hours post infestation. Samples of 30µl were loaded in each lane (A: fat body samples; B: haemolymph samples; Bf : *Busseola fusca*, /Av: parasitized by avirulent line of *C. sesamiae* from Mombasa; /V: parasitized by virulent line of *C. sesamiae* from Kitale; /NP: non parasitized larvae; LWM: low weight molecular standard; HWM: high weight molecular standard).

tissues were homogenised with a tissue grinder before they were prepared for loading. SDS-PAGE was carried out according to the method of Laemmli (1970) using 4-20 % (w/v) acrylamide gels which were run by loading 30 µl of each sample alongside 30 µl high and low molecular weight standards (Amersham Biosciences). A negative control was run on samples from *B. fusca* larvae that were not parasitized while a positive control was run on samples that were derived from the permissive host, *S. calamistis*. Gels were stained by the Coomassie Brilliant R250 and destained at room temperature.

Data analysis and interpretation

Silver stained 2-D gels spots were compared by superimposing the corresponding Kitale and Mombasa gels on a light box. We considered differences in spots either present or absent in gels of samples run on the 2-D gel electrophoresis. Only distinct spots were considered. For the haemolymph and fat body samples, specific bands on the coomassie stained SDS gels were

compared with the control and bands by the permissive host *S. calamistis*. Scoring was done qualitatively for bands that were present in the test samples but not in the control. Both the 2-D gels and the SDS gels were repeated at least 3 times. Only repeatable qualitative differences were considered.

Results

2-D gels protein migration patterns for calyx fluid samples extracted from the virulent Kitale and avirulent Mombasa *Cotesia sesamiae* are shown in fig. 2. Sustainable differences were observed between the two biotypes. There were more protein spots in protein gels with calyx fluid samples from virulent *C. sesamiae* biotype compared to the avirulent biotype (Chi sq = 7.00; df = 1; P = 0.0082). There were nine virulent specific spots present in Kitale calyx-fluid gels,
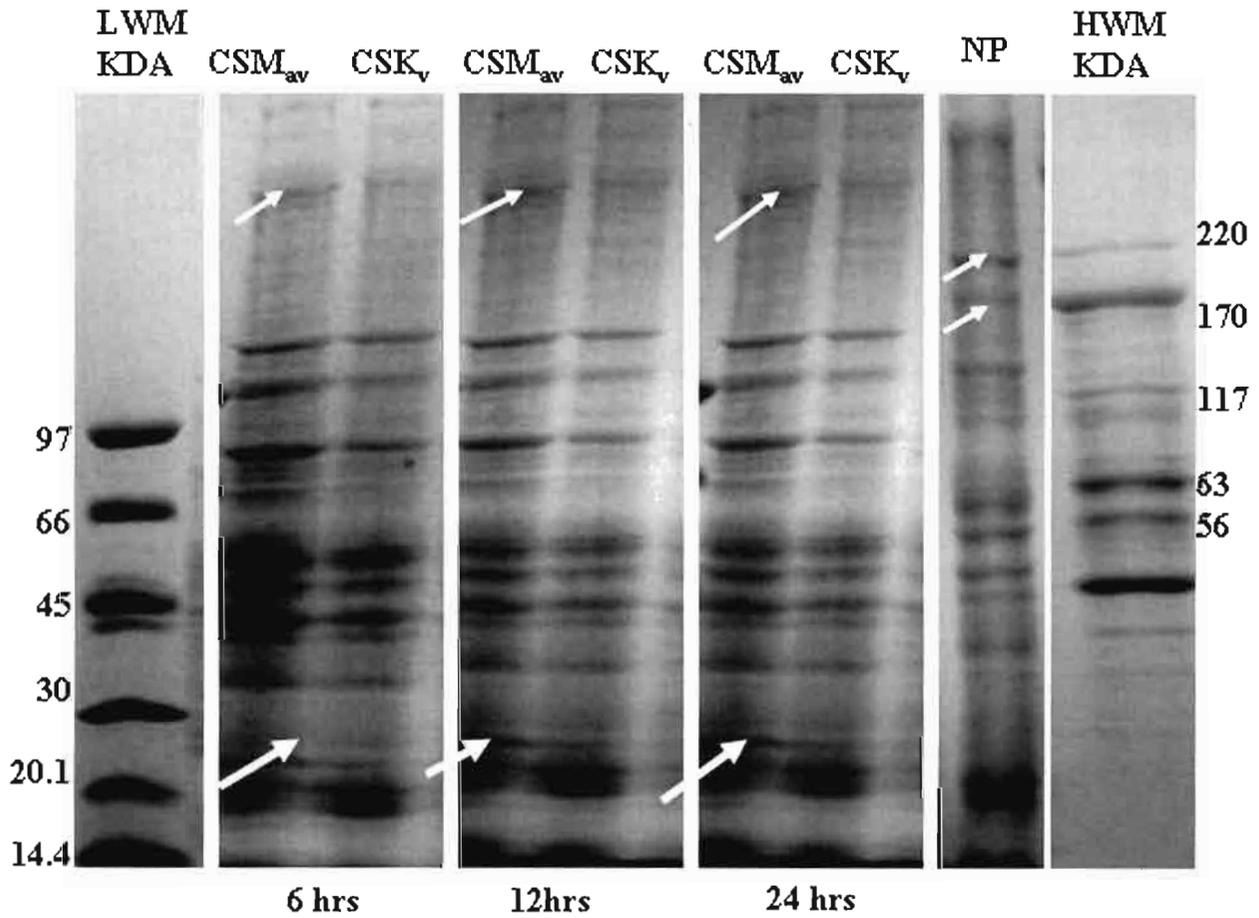
Figure 4
SDS-PAGE migration (4-20% gradient) of proteins from *Sesamia calamistis* and *Busseola fusca* tissues dissected from larvae parasitized by virulent (V) and avirulent (Av) lines of *C. sesamiae* at 12 hours post-infestation.30µl of samples were loaded in each lane. Bf : *B. fusca*, Sc: *S. calamistis*, /Av: parasitized by avirulent line of *C. sesamiae* from Mombasa, /V: parasitized by virulent line of *C. sesamiae* from Kitale, /NP: nonparasitized. LWM: low weight molecular standard. HWM: high weight molecular standard in kilodaltons (A: fat body samples; B: haemolymph samples; Bf : *Busseola fusca*; /Av: parasitized by avirulent line of *C. sesamiae* from Mombasa; /V: parasitized by virulent line of *C. sesamiae* from Kitale; /NP: non parasitized larvae; LWM: low weight molecular standard; HWM: high weight molecular standard in Kilodaltons).

5 avirulent-specific spots present in Mombasa calyx-fluid gels, and five spots were present in both gels.

SDS-PAGE gels were compared for haemolymph and fat body (fig. 3, 4). The bands scored are shown in tab. 1 and tab. 2. The intensity of bands increased with time post oviposition for the bands scored for tissues parasitized by the two *C. sesamiae* strains. Bands were always more intense for fat bodies from larvae parasitized by virulent *C. sesamiae* females than for fat bodies from larvae parasitized by avirulent *C. sesamiae* females. There were more bands on fat bodies compared to haemolymph samples signifying more complex expression in the fat bodies than in the haemolymph. Only two protein bands, at >220 and 28 Kb were scored for haemolymph from larvae parasitized by avirulent *C. sesamiae*. These bands were absent in the control samples signifying that these proteins were induced by parasitism by the avirulent *C. sesamiae* strain.

Protein bands compared between permissive (*S. calamistis*) and non-permissive (*B. fusca*) host species from the fat body and haemolymph samples parasitized with the virulent and avirulent *C. sesamiae* strains (figs. 5 and 6 respectively). The marked bands present on the permissive host were similar for larvae parasitized by both strains, for both fat body and haemolymph samples. This is consistent with the hypothesis that the proteins induced by parasitism in permissive hosts do not depend on the virulence to non permissive host of the parasitoid strain.

Discussion

In host insects with non-cellular defence capacities, additional strategies are required to completely protect the parasitoid against the host defence reactions. As bracoviruses are released from calyx cells by a lysis process, it is possible that non-assembled virus proteins are present in the calyx fluid. Eggs that pass through the calyx gland are exposed to components from the fluid and some of the proteins might become attached to the eggs surface offering it protection before PDV expression begins. Studies with *Cotesia rubecula* show that dissected eggs from the ovaries get encapsulated whereas eggs from the calyx gland and in the oviduct are protected (Asgari & Schmidt 1994b). This indicates that the protective layer is acquired within the calyx gland as the egg passes from the ovary to the oviduct. It is speculated that the proteins also protect the intact virus from recognition by the host.

Virulent *Cotesia sesamiae* populations showed marked differences in the proteins present in the calyx fluid compared to avirulent *C. sesamiae* population. The avirulent strain is lacking two particular spots present in the Kitale *C. sesamiae* strain, one of 40 kDa, another of 32 kDa. It can be speculated that these different spots may play a role in immune suppression since they are absent in the avirulent strain. Common protein spots present in the two strains can exhibit amino acid substitution, leading to a non functional protein in avirulent line in *Busseola fusca* host. Alternatively, common proteins are not involved in the variations between the strains.

Table 1. Analysis of fat body samples at different time points compared with the control samples for selected bands obtained wirh SDS-PAGE. CSM=*Cotesia sesamiae* from Mombasa, CSK=*Cotesia sesamiae* from Kitale. av and v=avirulent and virulent respectively.

Analyzed bands Molecular weight in KDA	NP	CSM$_{av}$ 6 h	CSK$_v$ 6 h	CSM$_{av}$ 12h	CSK$_v$ 12 h	CSM$_{av}$ 24 h	CSK$_v$ 24 h
>>220	0	*	**	*	**	**	***
>220	0	0	*	*	**	*	*
40	0	0	0	0	*	0	0
32	0	0	0	0	*	0	*
28	*	*	*	**	0	0	0

Amplification of the band pattern by SDS-PAGE on the gel. *** = Strong; ** =Moderate; * = Slight; 0 = No band; av=avirulent; v=virulent.

Table 2. Analysis of haemolymph at different time points compared with the control samples for selected bands obtained with SDS-PAGE. CSM=*Cotesia sesamiae* from Mombasa, CSK=*Cotesia sesamiae* from Kitale. av and v=avirulent and virulent respectively.

Analyzed bands Molecular weight in KDA	NP	CSM$_{av}$ 6 h	CSK$_v$ 6 h	CSM$_{av}$ 12 h	CSK$_v$ 12 h	CSM$_{av}$ 24 h	CSK$_v$ 24 h
>220	0	0	*	*	**	*	*
28	0	**	*	**	*	**	*

Amplification of the band pattern by SDS-PAGE on the gel. *** = Strong; ** =Moderate; * = Slight; 0 = No band; av=avirulent; v=virulent.

A 32-kDa protein (Crp32) and a heat-shock proteins CrHs70 and calreticulin CrCRT have been implicated in the prevention of cellular encapsulation of *C. rubecula* eggs in *Pieris rapae* (Asgari *et al.* 2003). Beckage *et al.* (1986) found that *Manduca sexta* (L. 1763) (Lepidoptera: Sphingidae) larvae naturally parasitized by *Cotesia congregata* (Say 1836) produced proteins of 56-kDA and 60-kDA whereas larvae injected with calyx cells (cells where PDV's are produced) extract, produced a 33-kDA polypeptide. The authors concluded that this polypeptide results from a viral gene expression or is a protein induced by presence of the virus. In *C. rubecula*, a non PolyDNAvirus 65-kDa protein reacted with specific antibodies similar in size to protein known to be involved in host immune suppression. In the present study, a 32-kDA and a 40-kDA protein were observed in fat body samples parasitized by the virulent strain at 12 and 24 hours but not on fat body samples parasitized by the avirulent strain. Proteins size greater that 220-kDA was also observed in the haemolymph and fat bodies larval samples parasitized by both the virulent and avirulent *C. sesamiae* strains. Grossniklaus-Burgin *et al.* (1998) observed a 212-kDA protein band in haemolymph of *Spodoptera littoralis* (Boisduval 1833) (Lepidoptera: Noctuidae) parasitized by *Chelonus inanitus* L. 1767 (Hymenoptera: Braconidae). This band was absent in the non-parasitized larvae.

To examine possible changes in the host organism after parasitization, protein extracts from haemolymph and fat bodies were analysed and compared to non-parasitized larvae. Results from this study provide some evidence that calyx fluid proteins or proteins expressed by polydnavirus may be capable of inducing significant physiological alterations, in *B. fusca* larvae as well as in the permissive host *Sesamia calamistis*. Compared to the control, some proteins seem to be inhibited when avirulent *C. sesamiae* Mombasa parasitizes *B. fusca* while others are enhanced in the fat body when the virulent *C. sesamiae* Kitale parasitizes the hosts. These alterations may be associated with suppression of host defence mechanism as suggested by other authors (Beckage *et al.* 1986; Stolz & Guzo 1986; Beckage & Kanost 1993; Strand & Noda 1991).

Fat bodies of larvae infested by the virulent strain had four different protein bands that were absent in the non-parasitized control. Fat bodies are likely to be the tissues in which the proteins are expressed, or changes within the host tissues are most noticeable. Obviously, parasitism has drastic physiological effects on this tissue which gradually atrophies after parasitoid eggs hatch and the host stops feedings. Although the fat body is not directly consumed by the wasp, it apparently experiences severe metabolic stress during the early and final stages of association of the endoparasitoids and their hosts. Encapsulated eggs can be observed mostly in the fat bodies 6 hours post parasitism (Ngi-Song *et al.* 1998; Gitau unpublished).

Host immune evasion in PDV containing wasps is likely mediated by expression of viral genes or host genes under the regulation of the PDV, calyx proteins or venom which may differ depending on the host. This is the first study that compared virulent and avirulent lines of parasitoids associated with PDVs for their calyx protein contents. The identification of differences opens new lines of researches. Two main issues deserve investigation. Are the proteins in the calyx fluid of *C. sesamiae* females related to viral proteins or do they get attached to the egg surfaces or the virus particles on their passage from the ovaries into the oviduct.

Conclusion

The differences in protein spots and bands show that there is variation in the two *Cotesia sesamiae* biotypes at the protein level. The virulent strain would be the best host to release or redistribute in areas where *Busseola fusca* is the dominant species. The avirulent strain would be best when released in areas where other stem borers are abundant. Studies to compare the fecundity of the virulent and avirulent strain are currently going on (Gitau *et al.* unpublished). Information gathered from both these studies will shed light into whether the avirulent strain is inferior to the virulent one and if it can be used for biological control where *B. fusca* is absent.

The present study indicates that the calyx proteins of virulent and avirulent strains are different, and that larvae parasitized by either the *C. sesamiae* strain elicits production of different proteins in the host which may affect development of the parasitoids larvae. Clarification of the origin of these proteins *i.e.* hosts versus parasitoid tissues and characterization of their biological role during parasitism remains a big challenge. Whether the differences in the protein patterns are responsible for the variation in the ability of *C. sesamiae* from Mombasa to develop in *B. fusca* still remain to be examined. Several proteins differ between virulent and avirulent parasitoid calyx fluids, suggesting virulence variations are governed by several factors, likely with epistatic interactions especially between viral and parasitoid proteins. Sequencing the bands, common or specific to each biotype would elucidate the role of calyx fluid proteins in host immune evasion. Eluting and injecting them in *B. fusca* would allow us to determine their respective function and potential

epistatic interactions. Ultimately, developing markers for all these factors would allow the survey of genetic adaptation of this parasitoid to its hosts in endemic regions, or in exotic areas, following its introduction for biological control.

References

Asgari S., Schmidt. O. 1994a. PolyDNAviruses produced in the calyx glands of *Cotesia rubecula* (Hymenoptera: Braconidae). *Journal of the Entomological Society of Iran* 14: 13-16.

Asgari S., Schmidt. O. 1994b. Passive protection of eggs from the parasitoid, *Cotesia rubecula*, in the host, *Pieris rapae*. *Journal of Insect Physiology* 40: 789-795.

Asgari S., Zhang G., Schmidt O. 2003. Polydnavirus particle proteins with similarities to molecular chaperones, heat-shock protein 70 and calreticulin. *Journal of General Virology* 84: 1165-1171

Beckage N.E., Templeton T.J., Nielsen B.D., Cook D.I., Stolz D.B. 1986. Parasitism-induced haemolymph proteins in *Manduca sexta* (L.) larvae parasitized by the Braconid wasp *Cotesia congregata* (Say). *Insect Biochemistry* 17: 439-455.

Beckage N.E., Kanost M.R. 1993. Effects of parasitism by the braconid wasp *Cotesia congregata* on host haemolymph proteins of the tobacco hornworm, *Manduca sexta. Journal of Insect Biochemistry and Molecular Biology* 23: 643-653.

Beckage N.E. 1998. Parasitoids and PolyDNAviruses. *Bioscience* 48: 305-310.

Belle E., Beckage N.E., Rousselte J., Poirié M., Lemeunier F., Drezen J.-M. 2002. Visualization of PolyDNAvirus sequences in a parasitoid wasp chromosome. *Journal of Virology* 76: 5793-5796.

Drezen J.-M., Huguet E., Dupuy C., Poirié M. 2000. PolyDNAviruses: how viruses evolve after domestication. *Research Advances in Virology* 1: 45-77.

Fleming J.A. 1992. PolyDNAviruses: Mutualists and pathogens. *Annual review of Entomology* 37: 401-423.

Grossniklaus-Burgen C., Pfister-Wlhelem R., Meyer V., Treiblmayr K., Lanzerin B. 1998. Physiological and endocrine changes associated with PolyDNAvirus/venom in the parasitoid-host system *Chelonus inanitus-Spodoptera littoralis. Journal of Insect Physiology* 44:305-321.

Hayakawa Y. 1994. Cellular Immunosuppressive protein in the plasma of parasitized insect larvae. *The Journal of Biological Chemistry* 269: 14536-14540.

Hayakawa Y., Yazaki, K. 1997. Envelope protein of parasitic wasp symbiont virus, PolyDNAvirus, protects the wasp eggs from cellular immune reactions by the host insect. *European Journal of Biochemistry* 246: 820-826.

Kimani-Njogu S. W., Overholt W. A. 1997. Biosystematics of the *Cotesia flavipes* complex (Hymenoptera: Braconidae) parasitoids of gramineous stem borers *Insect Science and its Application* 17: 119-130.

Laemmli U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.

Mochiah M.B., Ngi-Song A.J., Overholt W.A., Botchey M. 2002. Effects of calyx fluid from two populations of *Cotesia sesamiae* on *Busseola fusca* (Lepidoptera: Noctuidae). *Insect Science and its Application* 21: 369-374.

Mohyuddin A.I. 1971. Comparative biology and ecology of *Apanteles flavipes* (Cam.) and *A. sesamiae* Cam. as parasites of graminaceous borers. *Bulletin of Entomological Research* 61: 33-39.

Mohyuddin A. I. 1990. Biological control of *Chilo* Spp. in maize and millet. *Insect Science and its Application* 11: 721-732.

Morrissey J.H. 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. *Analytical Biochemistry* 117: 307-310.

Ngi-Song A.J., Overholt W.A., Stouthamer R. 1998. Suitability of *Busseola fusca* and *Sesamia calamistis* (Lepidoptera: Noctuidae) for the development of two populations of *Cotesia sesamiae* (Hymenoptera: Braconidae) in Kenya. *Biological control* 12: 208-214.

O'Farrell P.H. 1975. High resolution two-dimensional electrophoresis of proteins. *Journal of Biochemistry* 250: 4007–4021.

Onyango F.O., Ochieng'-Odero J.P.R. 1994. Continuous rearing of the maize stem borer, *Busseola fusca* on a semi-synthetic diet. *Entomologia Experimentalis et Applicata* 73: 139-144.

Overholt A.W., Ochieng J.O., Lammers P., Ogedah K. 1994. Rearing and field releases methods for *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stem borers. *Insect Science and its Application* 15: 253-259.

Polaszek A., Walker A.K. 1991. The *Cotesia flavipes* species-complex: Parasitoids of cereal stem borers in the tropics. *Redia* 74: 335-341.

Ratcliffe N.A. 1993. Cellular defence responses of insects: Unresolved problems, p. 267-304 in : Beckage N., Thompson S., Federici B. (ed.), *Parasites and pathogens of insects*. Academic press, San Diego.

Savary S., Beckage N., Tan F., Periquet G., Drezen J.M. 1997. Excision of the PolyDNAvirus chromosomal EPI sequence of the parasitoid *Cotesia congregata* (Braconidae: Microgasterinae) at potential recombinase binding sites. *Journal of General Virology* 78: 3125-3134.

Schulthess F., Bosque-Perez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Goergen G. 1997. Exchange of natural enemies of lepidopteran cereal stem borers between African regoins. *Insect Science and its Application* 17: 97-108.

Stolz D.B. 1986. Interactions between parasitoid-derived products and host insects: An overview. *Journal of Insect Physiology* 32: 347-350.

Stolz D.B., Guzo D. 1986. Apparent haemocytic transformation associated with parasitoid-induced inhibition of immunity in *Malacosoma disstria* larvae. *Journal of Insect Physiology* 32: 377-388.

Stolz D.B. 1992. Viruses and virus like entities in the parasitic Hymenoptera. *Journal of Hymenoptera research* 1:125-139.

Strand M.R., Noda T. 1991. Alterations in the haemocytes of *Pseudoplusia includens* after parasitism by *Microplitis demolitor. Journal of Insect Physiology* 37:503-515.

Theilmann D.A., Summers M.D. 1986. Molecular analysis of *Campoletis sonorensis* virus DNA in the lepidopteran host *Heliothis virescens. Journal of General Virology* 67: 1061-1969.

Vinson S.B. 1990. How parasitoids deal with the immune system of their host: An overview. *Archives of Insect Biochemistry and Physiology* 13: 3-27.

Webb B.A., Luckhart S. 1994. Evidence for an early immunosuppressive role for related *Campoletis sonorensis* venom and ovarian proteins in *Heliothis virescens. Archives of Insect Physiology and Biochemistry* 26: 147-163.

A model for the study of *Wolbachia pipientis* Hertig (Rickettsiales: Rickettsiaceae)- induced cytoplasmic incompatibility in arrhenotokous haplodiploid populations: consequences for biological control

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Abstract. *Wolbachia* is an endocytoplasmic bacterium responsible for various reproductive modifications in arthropods. In several species, *Wolbachia* induces a phenomenon called cytoplasmic incompatibility (CI), whereby crosses between a *Wolbachia*-infected male and a healthy female are incompatible. In haplodiploid species reproducing with arrhenotokous parthenogenesis, CI crosses produce only parthenogenetic males, inducing a male-biased sex ratio in the population. Here, we used two modeling approaches to evaluate the respective influences of demographic and biological parameters on *Wolbachia* fixation probability and on the sex ratio peak occurring during a *Wolbachia* invasion, and compared these parameters to values reported in the literature. Results suggest that the impact of *Wolbachia* invasion on population dynamics remains relatively limited, especially for parasitoids with high rates of sib-mating. The consequences for introduction of the parasitoids for biological control are discussed.

Résumé. Un modèle d'étude de l'incompatibilité cytoplasmique induite par *Wolbachia pipientis* Hertig 1936 (Rickettsiales : Rickettsiaceae) induisant une incompatibilité cytoplasmique chez les populations arrhénotoques hapodiploïdes : les conséquences pour la lutte biologique. *Wolbachia* est une bactérie endocytoplasmique responsable de plusieurs phénomènes de modification de la reproduction. Chez plusieurs espèces, *Wolbachia* induit un phénomène appelé Incompatibilité Cytoplasmique (IC) : les croisements entre mâle infecté par *Wolbachia* et femelle non-infectée sont incompatibles. Chez les espèces incompatibles se reproduisant par parthénogénèse arrhénotoque, du fait que les croisements incompatibles donnent uniquement des mâles, l'IC entraîne un biais de sex-ratio dans la population. Dans cette étude, nous avons utilisé deux approches de modélisation pour évaluer les influences respectives de paramètres démographiques et biologiques sur la probabilité de maintenir *Wolbachia* et sur l'augmentation de la sex-ratio durant la phase d'invasion. Ces paramètres ont été comparés aux valeurs observées dans la littérature. Les résultats suggèrent que l'impact de l'invasion de *Wolbachia* sur la dynamique des populations est relativement limité, particulièrement pour les parasitoïdes avec un fort taux de croisement frères-soeurs. Les conséquences pour les stratégies d'introduction de parasitoïdes dans le cadre de la lutte biologique sont discutées.

Keywords: *Wolbachia*, stochastic modeling, biological control, stem borer, Africa.

Many microorganisms, including bacteria (Hunter 1999) and viruses (Varaldi *et al.* 2003), can modify the reproductive strategies of insects. Such effects can have demographic consequences on beneficial insects including the parasitoid wasps commonly used as classical biological control agents. Understanding these constraints may allow a better biological control strategy for controlling pests.

Wolbachia pipientis Hertig 1936 (Rickettsiales: Rickettsiaceae) is an endocytoplasmic symbiotic bacterium responsible for many reproductive modifications in arthropods and other phyla (Werren 1997). In several species, these bacteria cause

cytoplasmic incompatibility (CI) (reviewed in Stouthamer *et al.* 1999) which affects population dynamics by preventing crosses between infected and healthy individuals. In haplodiploid species reproducing with arrhenotokous parthenogenesis, such as parasitoid wasps, incompatible crosses occurring between infected males and healthy females leads to male progeny only. This can result from two different CI phenotypes affecting diploid eggs. In the case of the Male Development (MD) phenotype, diploid eggs develop as males, whereas in the case of the Female Mortality (FM) phenotype, diploid eggs die (Vavre *et al.* 2000). Both MD and FM phenotypes lead to a male-biased sex ratio in the population. Since only females lay eggs, population growth is particularly correlated to female frequency.

Population genetic diversity may also be affected by CI: in gregarious parasitoids, siblings often mate

together after hatching from cocoon masses, but cocoons affected by CI will produce only males and will not be able to sib-mate, thereby reducing population inbreeding. Thus, *Wolbachia* could potentially affect population genetics and dynamics during an invasion by reducing the number of females. The theoretical model proposed by Mochiah and co-workers (2002) showed that introducing a biocontrol agent infected by *Wolbachia* into a non-infected population results in a transient reduction in population growth rate due to *Wolbachia* invasion. This model considered the effect of sib-mating, but did not consider the effect of other parameters varying between species and/or the *Wolbachia* strains and density (Guillemaud *et al.* 1997) as penetrance of CI (the proportion of crosses predicted to be incompatible that are really incompatible), and the rates of vertical transmission of *Wolbachia* (the probability for a female to transmit *Wolbachia* to its offspring) (Turelli & Hoffmann 1995; Rasgon & Scott 2003); such parameters may affect the impact of *Wolbachia* on population growth rate. Furthermore, this model is determinist and does not consider the effect of population size.

Here, we evaluate the robustness of the model developed by Mochiah *et al.* (2002) on the demographic impact of *Wolbachia* invasion in a resident population of a parasitoid over a wider range of parameters. Some effects on genetic diversity are also evaluated. Hypotheses of stochastic dynamics and finite population size, which are likely to be more realistic, are considered. The effects of variations in CI penetrance and rates of vertical transmission on the sex ratio modification and on the probability of *Wolbachia* fixation are also evaluated.

First the discrete determinist equations are used as in Mochiah *et al.* (2002) and Stouthamer *et al.* (2000) to show the impact of several life history traits on *Wolbachia*-parasitoid dynamics. Then the results are compared to the stochastic model with finite population size.

Material and methods

Equations

Equations were developed based on Mochiah *et al.* (2002) and Stouthamer *et al.* (2000) to determine the evolution of sex ratio (proportion of males) during *Wolbachia* invasion. We considered two additional parameters: penetrance of CI and *Wolbachia* vertical transmission rate (see above).

Assuming infinite population size and no migration. $F_{I,T}$ and $M_{I,T}$ are the proportion of infected females and males at time t, respectively. Other parameters were x, the proportion of females in the offspring; tCI, the penetrance of CI; μ the vertical transmission rate; and s, the sib-mating frequency.

At $t = 0$, 10 percent of the population is infected. The sex ratio (males:females) is given a value of $x = 0.25$ in the absence of a *Wolbachia* effect. Local mate competition leads to female-biased sex ratios, because it is advantageous for a female to produce more females when the number of competitor females is low (Hamilton 1967). Furthermore, in arrhenotokous haplodiploids species, females can easily control the progeny sex ratio because males result from unfertilized eggs (Hardy *et al.* 1999). We chose to apply a female-biased sex ratio (i.e. 0.25) as observed, for instance, in *Cotesia sesamiae* (Cameron 1891) (Hymenoptera: Braconidae) (Le Rü *pers. com.*), a biological control agent used against stemborers in Africa.

The proportion of infected females from one generation to the next is given by the equation below; note that it does not depend on the nature of the CI phenotype, MD or FM:

$$F_{I,T+1} = \frac{\mu F_{I,T}}{s + (1-s)} \times$$

$$\times \frac{1}{[F_{I,T} + (1 - F_{I,T})(1 - M_{I,T}) + (1 - tCI)(1 - F_{I,T})M_{I,T}]}$$

The number of infected males in the next generation depends on the CI phenotype. Under FM, it is:

$$M_{I,T+1} = \mu F_{I,T}$$

Under the MD phenotype, incompatible crosses produce fewer infected males:

$$M_{I,T+1} = \frac{\mu F_{I,T}(1-x)}{(1-x) + x(1-s)(1-F_{I,T})M_{I,T}tCI} \quad (3)$$

Assuming x is constant between generations, the sex ratio in the population at each generation is given for the FM phenotype by:

$$SR_{T+1} = x\frac{1 - (1-s)(1-F_{I,T})M_T tCI}{1 - x(1-s)(1-F_{I,T})M_T tCI} \quad (4a),$$

and for the MD phenotype by:

$$SR_{T+1} = x[1 - (1-s)(1-F_{I,T})M_{I,T}tCI] \quad (4b).$$

Sex ratio variations were calculated among generations assuming equation 4 for sets of values of s, μ and tCI.

Stochastic model

The stochastic model was implemented under Scilab 3.1 (INRIA 2005). Individuals were identified by infection status (TRUE for infected and FALSE for uninfected). We assumed 10 percent of the population was infected at $t = 0$, and a population size that was constant among generations. The number of females ($Nf_{T,t}$) and males ($Nm_{T,t}$) were calculated as a function of the sex ratio. $Nf_{I,t}$ and $Nm_{I,t}$ are the number of infected females and males, respectively, at time t. Within the total populations, the infected effective (reproductive) females and males (Nf'_I and Nm'_I, respectively) are randomly sampled, assuming the number of infected females and males is given by:

$$Nf_I^{'} \to B(Nf_t, \frac{Nf_{I,t}}{Nf_{T,t}}) \quad \text{(Ia), and}$$

$$Nm_I^{'} \to B(Nm_t, \frac{Nm_{I,t}}{Nm_{T,t}}) \quad \text{(Ib)},$$

where $B(N,p)$ is the binomial probability distribution of probability p and number of trials N.

Infected male and female reproducers ($Nf_I^{''}$ and $Nm_I^{''}$, respectively) are sampled with a fitness-cost probability $1-c$, where c represents the fitness cost of carrying *Wolbachia* :

$$Nf_I^{''} \to B(Nf_I^{'},1-c) \quad \text{(IIa)}$$

$$Nm_I^{''} \to B(Nm_I^{'},1-c) \quad \text{(IIb)}$$

Mating was independent of infection status. Sib-mating females ($Nf_{I,S}$) were randomly sampled with a probability s:

$$Nf_{I,S} \to B(Nf_I^{''},s) \quad \text{(III)}.$$

Infected females after reproduction ($Nf_I^{'''}$) are sampled assuming a vertical transmission probability μ:

$$Nf_I^{'''} \to B(Nf_{I,S},\mu) + B(Nf_I^{''} - Nf_{I,S},\mu) \quad \text{(IV)}.$$

The number of healthy females that do not sib-mate ($Nf_{H,I-S}$) that mate of them mating with infected males (N_{CI}) were sampled assuming a sib-mating probability s and proportion of infected males sampled above :

$$Nf_{H,1-S} \to B(1-Nf_I^{'''},1-s) \quad \text{(Va), and}$$

$$N_{CI} \to B(Nf_{H,1-S}, \frac{Nm_I^{'''}}{Nm_I + Nm_H}) \quad \text{(Vb)}.$$

Effective CI matings ($N_{CI,t}$) are sampled from N_{CI} with a

$$N_{CI,t} \to B(N_{CI},tCI) \quad \text{(VI)}.$$

penetrance tCI (CI penetrance):
The total number of females and males at $t+1$ was calculated as:

$$Nf_{T,t+1} = Nf_{T,t} - N_{CI,t} \quad \text{(VIIa)}$$

$$Nm_{T,t+1} = Nm_T \quad \text{(VIIb)}$$

under the FM phenotype, or

$$Nm_{T,t+1} = Nm_T + N_{CI,t} \quad \text{(VIIc)}$$

under the MD phenotype.

The sex ratio of the next generation is given by:

$$SR_{t+1} = \frac{Nm_{T,t+1}}{Nf_{T,t+1} + Nm_{T,t+1}} \quad \text{(VIII)}.$$

When a parameter varies, sib-mating is fixed at 0.5, tCI and μ are fixed at 1, physiological cost is fixed at 0 and population size is fixed at 200 individuals.

The sex ratio (proportion of males) and the probability that *Wolbachia* is maintained in the population after 40 generations, Pm, (number of infected populations after 40 generations divided by number of repetitions) were recorded. Statistics were performed with R Development Core Team (2005) (version 2.2.0).

Results

The impact of the FM phenotype on sex ratio is weaker and shorter in duration than that of MD (fig. 1), but their response curve to variations in parameter values have the same general shape. The results are presented below for the MD phenotype only.

Comparison of stochastic and determinist models (fig. 2)

Almost the same results were found between stochastic and determinist models for the effect of invasion on sex ratio. The major difference was that *Wolbachia* invasion was faster in the stochastic case because of the drift effect. Between different stochastic models, the smaller the population, the faster is the invasion. Another difference between the stochastic and determinist models are estimates of a population's ability to maintain a *Wolbachia* infection. In the case of the determinist model, the population cannot maintain *Wolbachia* below a transmission rate of 0.97, while in the stochastic model *Wolbachia* can be maintained for transmission rates less than 0.9, due to drift.
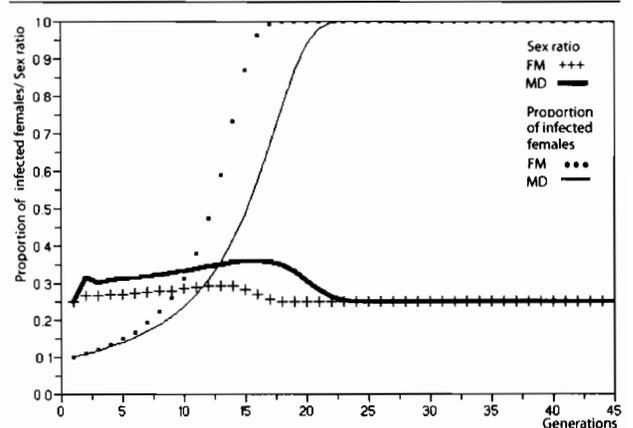

Figure 1
Evolution of sex-ratio and proportion of parasitoid females infected with *Wolbachia* in Female Mortality phenotype (FM) and Male Development phenotype (MD) ($s = 0.5$, $tCI = 1$, $\mu = 1$).

Sib-mating (fig. 2)

As in Mochiah *et al.* (2002), sib-mating strongly reduces the sex ratio peak during invasion. This is due to a decrease in the proportion of mating between infected and non-infected individuals. We also found that sib-mating at high rates reduces the probability of maintaining *Wolbachia*. This is due to an increase in the drift effect, which reduces the effect of *Wolbachia* fitness advantage (only infected females reproduce) during invasion.

CI penetrance (fig. 2)

A lower CI penetrance has a similar but less important impact on the sex ratio than a higher rate of sib-mating. As for sib-mating, a lower CI penetrance reduces the effect on the sex ratio, but by slowing down the invasion process, it distributes the sex ratio impact among more generations.

Transmission rate (fig. 2)

The transmission rate does not affect the sex ratio disturbance but greatly affects the probability of maintaining *Wolbachia*. For instance, in a population size of 200 individuals, if the probability of vertical transmission is 87%, the Pm drops to 0.040 ± 0.058 (95% confidence interval). *Wolbachia* needs a strong vertical transmission rate in CI to be maintained in the population.

Physiological cost (fig. 3)

As for the transmission rate, the physiological cost does not affect the sex ratio disturbance but it does affect the probability of maintaining *Wolbachia*. This means that the selective advantage of infected females in comparison to healthy ones is rapidly balanced by the physiological cost of carrying the bacteria.
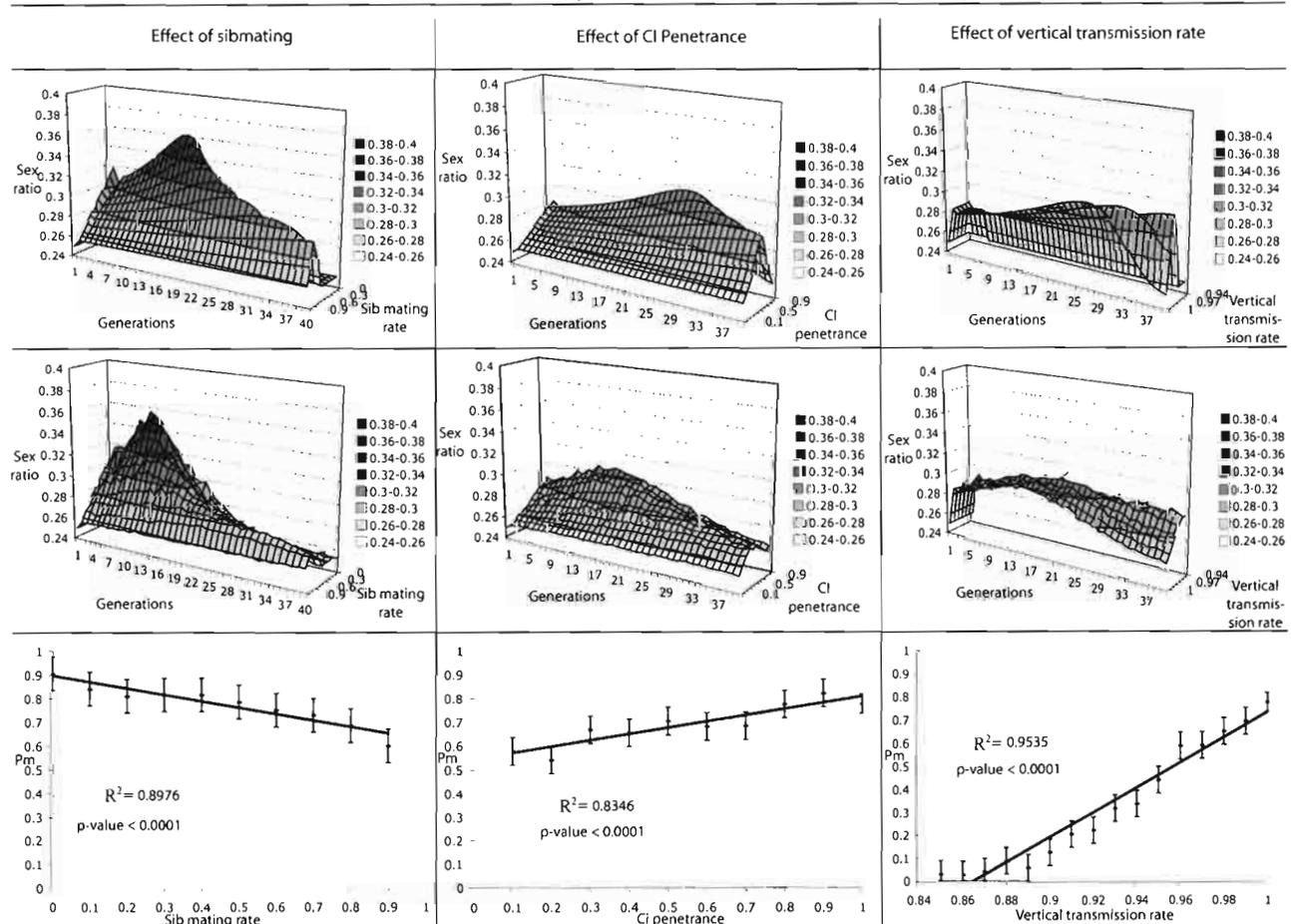
Figure 2
Effects of parameters on sex ratio and probablity of maintaining *Wolbachia* infection in a population of parasitoids: (a) sex ratio results (proportion of males) in the stochastic model, (b) sex ratio results in the determinist model, (c) probability of maintening *Wolbachia*. In 3D graphics (a and b), X-axis represents time in generations, Y-axis represents sex ratio value and Z-axis correspond to values of the variables (sib-mating, CI (cytoplsmic incompatability) penetrance or transmission rate). In 2D-graphics (c), Y-axis corresponds to probability of maintaining *Wolbachia* in the population and X-axis to values of the variables.

Population size (fig. 3)

Population size has an effect on Pm up to $N = 500$ (Pm = 0.95 ± 0.07). Invasion time is longer when the population is larger.

Discussion

The two *Wolbachia* invasion models developed in this work show that the sex ratio during invasion is always less than 50%, suggesting that a viable population could overcome the detrimental effects of *Wolbachia* invasion. In addition, it is observed that several life history traits can reduce the effect of *Wolbachia* invasion on population dynamics. A high sib-mating rate or a low CI penetrance limits the impact of *Wolbachia*. However, for transmission rates less than 1, infected and healthy individuals may coexist in the population, thereby leading to **invasion/loss** *Wolbachia* dynamics due to migration/drift processes with their associated detrimental effect on population growth rate.

In gregarious parasitoids, sib-mating occurs frequently. In *Cotesia glomerata* (L. 1758) (Hymenoptera: Braconidae), approximately 60% of females breed with their brothers (Kitano & Tagawa 1981). Thus, sib-mating could be an important limiting factor to the impact of *Wolbachia* invasion on population growth rate.

Previous investigations have shown that *Wolbachia* is transmitted at variable rates among different host species. In *Drosophila simulans* Sturtevant 1919 (Diptera: Drosophilidae), field data show a transmission rate of 96–97% (Turelli & Hoffmann 1995) and in the mosquito *Culex pipiens* L. 1758 (Diptera: Culicidae), a 98.6% transmission rate was reported (Rasgon & Scott 2003). Such high rates of transmission of *Wolbachia* in populations harbouring the bacterium are consistent with the prediction of the models presented in this work: when the bacterium induces CI, only a high rate of transmission will allow *Wolbachia* to be maintained within a population.
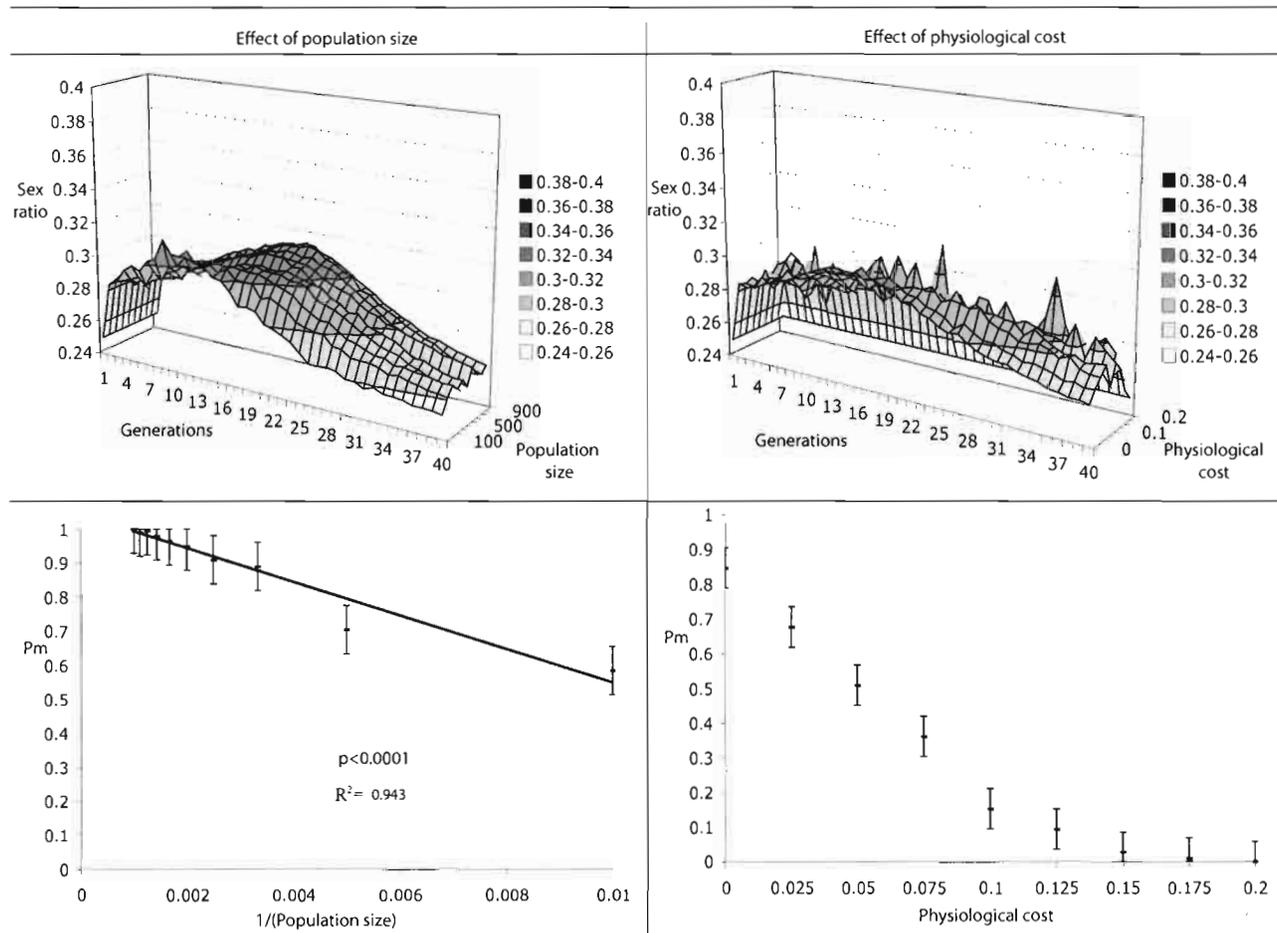
Figure 3
Effect of population size (a) and physiological cost (b) on sex ratio and probability of maintaining *Wolbachia* in parasitoid populations of different sizes (c) and physiological cost (d). For axes, see fig. 1.

Concerning the penetrance of CI, its value depends on several factors: the strain of *Wolbachia*, the genotype of the host and the density of bacteria in the eggs (Hunter 1999). Consequently, although it is difficult to estimate this parameter, we observed that it can have a strong effect on limiting invasion impact. We also found that *Wolbachia* must have a very limited or no fitness cost to be maintained in the population. The impact of *Wolbachia* on its host's fitness has been found to be very limited so far (Poinsot & Merçot 1997; Werren 1997; Stouthamer *et al.* 1999). Thus, it seems to be essential for this reproductive parasite to avoid any physiological cost to its host.

Populations of parasitoids may suffer important seasonal reductions in population size due to climatic factors or variations in host occurrence. Our model predicts that this situation would accelerate invasion.

In biological control, *Wolbachia* could play a major role if an infected population is introduced into a healthy one or the reverse. Since only the non-infected females suffer reproductive depression, the implications for introduction success differ between these two cases. The *Wolbachia*-free population will have a reduced growth rate, especially when less abundant. If the *Wolbachia*-free population is local, it may be endangered when the introduced population reaches high levels. If a *Wolbachia*-free parasitoid population is introduced into an infected population, it may have a reduced growth rate during the early stages of invasion when its population is low, which may compromise introduction success. Nevertheless, high sib-mating, as well as low CI penetrance, reduces this impact.

From the results of their model, Mochiah and co-workers (2002) have suggested that releasing individuals with *Wolbachia* status different from that of the native population may reduce the chance of introduction success, due to the impact of cytoplasmic incompatibility on the sex ratio. Our results suggest, however, that the effect of the sex ratio on overall reproductive rate (of introduced and local populations) may be limited. Nevertheless, *Wolbachia* can still have a strong effect on biological control introduction success in a situation in which the two populations are infected by *Wolbachia* from different strains which are reciprocally incompatible, thereby leading to bi-directional cytoplasmic incompatibility (BCI) (Bordenstein *et al.* 2001). In this case, CI is expressed in both directions of the cross. Therefore, an introduction of a population carrying a strain different from the native population could potentially affect the success of biological control more than in the other cases described previously. In such a case, the best solution may be to breed native populations in the laboratory and release them in their endemic location (augmentative biological control).

In conclusion, it is clear that before any use of a biological control agent infected with *Wolbachia*, the *Wolbachia* status of the local population must be known. While the predictive effect of *Wolbachia* is very difficult to estimate because of the difficulty associated with estimating some of the model parameters, our results show that in the presence of only one strain of *Wolbachia*, its effect on the success of a biological control program can be overcome.

References

Bordenstein S. R., O'Hara F. P., Werren J.H. 2001. Wolbachia-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature* 409(6821): 707-710.

Guillemaud T., Pasteur N., Rousset F. 1997. Contrasting levels of variability between cytoplasmic genomes and incompatibility types in the mosquito *Culex pipiens*. *Proceedings in Biological Science* 264: 245-251.

Hamilton W. D. 1967. Extraordinary sex ratios. A sex-ratio theory for sex linkage and inbreeding has new implications in cytogenetics and entomology. *Science* 156: 477-488.

Hardy I. C., Pedersen J. B. Sejr M.K., Linderoth U.H. 1999. Local mating, dispersal and sex ratio in a gregarious parasitoid wasp. *Ethology* 105: 57-72.

Hunter M. S. 1999. Genetic conflict in natural enemies: a review, and consequences for the biological control of arthropods, p. 231-258 *in*: Hawkins B. A., Cornell H. V. (eds.), *Theoretical Approaches to Biological Control*. Cambridge University Press, Cambridge.

INRIA 2005. *Scilab*, INRIA.

Kitano H., Tagawa J. 1981. Mating behaviour of the braconid wasp *Apanteles glomeratus* in the field. *Applied Entomology and Zoology* 16: 345-360.

Mochiah M.B., Ngi-Song A.J., Overholt W.A., Stouthamer R. 2002. *Wolbachia* infection in *Cotesia sesamiae* (Hymenoptera: Braconidae) causes cytoplasmic incompatibility: implications for biological control. *Biological Control* 25: 74-80.

Poinsot D., Merçot H. 1997. *Wolbachia* infection in *Drosophila simulans*: does the female host bear a physiological cost? *Evolution* 51: 180-186.

Rasgon J. L., Scott T. W. 2003. Wolbachia and cytoplasmic incompatibility in the California Culex pipiens mosquito species complex: parameter estimates and infection dynamics in natural populations. *Genetics* 165(4): 2029-2038.

R Development Core Team 2005. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Stouthamer R., Breeuwer J. A., Hurst G.D. 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Review of Microbiology* 53: 71-102.

Stouthamer R., Jochemsen P., Platner G.R., Pinto J.D. 2000. Crossing incompatibility between *Trichogramma minutumi* and *T. platneri* and its implication for their application in biological control. *Environmental Entomology* 29: 832-837.

Turelli M., Hoffmann A.A. 1995. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics* 140: 1319-1338.

Varaldi J., Fouillet P. Ravallec, M., Lopez-Ferber M., Bouletreau M., Fleury F. 2003. Infectious behavior in a parasitoid. *Science* 302: 1930.

Vavre F., Fleury F., Varaldi J.P., Fouillet P., Bouletreau M. 2000. Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic and evolutionary consequences. *Evolution* 54: 191-200.

Werren J.H. 1997. Biology of *Wolbachia*. *Annual Review of Entomology* 42: 587-609.

Vetiver grass (*Vetiveria zizanioides* (L.) Nash) as trap plant for *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae)

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Abstract. The preference of lepidopterous stem borer moths to oviposit on certain wild host plants can be exploited in habitat management systems by using those hosts as trap crops. Vetiver grass (*Vetiveria zizanioides* (L.) Nash) was evaluated for its attractiveness and suitability to the pyralid *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) and the noctuid *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). Two choice tests were conducted in the laboratory and in the greenhouse to determine oviposition choice of *C. partellus* for maize, Vetiver and rice (*Oryza sativa* L.), and of *B. fusca* for Vetiver and maize. *C. partellus* larval survival was evaluated in green house studies. Results indicated that *C. partellus* chose Vetiver grass over maize though larval survival on Vetiver was extremely low. *B. fusca* did not show any host preference.

Résumé. Le Vetiver (*Vetiveria zizanioides* (L.) Nash), plante piège pour *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) et *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae). La préférence des lépidoptères foreurs de graminées à pondre sur certaines gaminées sauvages plutôt que sur celles cultivées peut être exploitée dans un système de pratique culturale en utilisant ces graminées sauvages comme plantes-pièges autour des cultures. L'attractivité du Vétiver (*Vetiveria zizanioides* (L.) Nash) sur la ponte de *Chilo partellus* (Swinhoe) de *Busseola fusca* (Fuller) a été testée, de même que sa faculté à permettre le développement de ces deux espèces. Des tests de choix binaire ont été menés en laboratoire et en serre pour déterminer la préférence de ponte de *C. partellus* pour le maïs, le Vétiver et le riz (*Oryza sativa* L.), et celle de *B . fusca* pour le Vétiver et le maïs. Les taux de survie des larves de *C. partellus* ont été évalués en serre. Les résultats ont montré que les femelles de *C. partellus* choisissent plutôt le Vétiver au maïs alors que le taux de survie larvaire y est extrêmement faible. *B. fusca* n'a montré aucune préférence de ponte vis-à-vis du Vétiver.

Keywords: habitat management, host plant selection, preference-performance hypothesis, rice, trap crops.

The principle of trap cropping rests on the fact that virtually all insects show a distinct preference for certain plant species, cultivars or a certain crop stage (Hokkanen 1991). Preferences of lepidopterous stem borers for graminaceous plants have been reported for *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) (van den Berg *et al.* 2001; van den Berg 2006; Khan *et al. in press*) and for *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) (van den Berg *et al. in press*). Napier grass (*Pennisetum purpureum* L.) and sorghum (*Sorghum* spp.) were recommended as trap plants around maize fields in habitat management systems for stem borers in East and Southern Africa. While the attractiveness of Napier grass for especially *C. partellus* have been shown, its effect as trap crop could possibly also be partly ascribed to its role as a barrier to moths infesting fields outside (van den Berg *et al. in press*). Reduction in stem borer infestation in maize fields surrounded by wild grasses have also been reported by Khan *et al.* (1997), Ndemah *et al.* (2002) and Midega *et al.* (2005). Napier grass and forage sorghum are however not always suitable to farming conditions in low rainfall areas, or in farming systems where forages are not important and where free grazing is practised. Alternative trap crops need to be identified that could be used in systems where the use of Napier grasses and forage sorghums is not feasible.

Vetiver grass (*Chrysopogon zizanioides* (L.) Roberty = *Vetiveria zizanioides* (L.) Nash) is a species that is used globally as soil erosion management tool and in sustaining agricultural productivity (Grimshaw 2003). Vetiver grass technology, in its most common form, is the establishment of a narrow (less than 1 m wide) live, stiff grass barrier, in the form of a hedge across the slope of the land (Grimshaw 2003). The easy availability of Vetiver grass and its presence on contours between crop fields in many African countries prompted investigation into the possibility of using this grass as a trap plant for stem borers in maize production systems. Stem borers have been reported to damage Vetiver grass in China where this grass is indigenous (Xinbao 1992).

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Paddy stem borers (*Chilo* spp.) have been reported to infest culms and midribs of leaves wherever Vetiver was planted in Southern China. An interesting observation was that the levels of mortality amongst stem borer larvae was high and in the worst case, approximately 39 % of Vetiver stems were damaged by the borer but no pupae were found (Xinbao 1992), indicating that larval survival in Vetiver is low. If the oviposition choice of stem borer moths for Vetiver grass is high, which seems to be suggested by these observations, the possibility exists that it could be used as trap plants around crops on which stem borers are a problem. The aim of this study was to determine the oviposition choice of *B. fusca* and *C. partellus* moths for Vetiver grass and maize and to determine the survival of larvae on this plant species.

Materials and methods

Laboratory trials: Two-choice bioassays

Oviposition choice tests were done in muslin cloth cages (50 x 55 x 75 cm). Each test was replicated three times. Cages (replicates) were placed in the centre of a dark room with two pots placed inside each cage. One pot contained a Vetiver plant with 4–6 tillers and the other one 4-week old maize plant. Maize of this age was used because *B. fusca* was previously observed to prefer four to five week old plants for oviposition (van Rensburg *et al.* 1989) while *C. partellus* infest plants of any age (Seshu Reddy *et al.* 1990; Singh & Sandhu 1978). Five pairs of adult moths were released into the centre of each cage and allowed to oviposit overnight before they were removed. Although this is a high number of moths, not all of them were expected to be of egg laying age since their mating status was not determined before collection. Moths were collected in cages on the evening of emergence from sorghum stalks collected from the field. Moths were therefore naive and had no oviposition experience. Since *C. partellus* is known to attack rice (Seshu Reddy 1990), a test was also conducted to determine the oviposition choice of moths when presented with rice and Vetiver plants. One rice variety (accession number 966) was used in this study. The procedure was identical to that described in the two-choice test with maize. The number of egg batches per plant, eggs per batch and mean numbers of eggs per plant on the different plant species were recorded and subjected to Student t-tests to determine if there were significant differences.

Greenhouse trials: Larval survival on potted plants

In this experiment larval survival of *C. partellus* was determined on Napier grass, Vetiver grass and maize plants growing in pots. The objective was to compare larval survival on maize with that on Vetiver and Napier grass, which is currently used in habitat management for control of maize stem borers (Midega *et al.* 2005; van den Berg *et al.* 2001). Pots were kept in a greenhouse. Each plant species was replicated ten times (10 pots) with one plant per pot in a completely randomised design. Each plant was infested with one egg batch in the black head stage when maize plants were four weeks old. Each egg batch contained 30 eggs. Eggs were produced by wild moths that were collected in cages in which sorghum stalks, collected from the field,

were kept. Plants were kept in a greenhouse at 25 (±2) °C and 50–60 % relative humidity for 28 days after which they were dissected to recover surviving larvae. The number of surviving larvae was calculated from the number of eggs put on plants. The assumption was made that egg hatch was 100% and the numbers of eggs was then taken to represent the number of first-instar larvae that there was on each plant after egg hatch. Larval survival was expressed as a percentage of larvae recovered after 28 days in relation to the number of first- instar larvae (number of eggs).

Data on mean percentage survival, calculated in terms of number of eggs put on each plant were subjected to ANOVA and means separated by means of the Tukey test.

Greenhouse trials: Two-choice bioassay

A two-choice experiment that involved more plants and larger cages was subsequently conducted in a commercial green house where plants were growing in the soil. The aim was to determine *C. partellus* oviposition choice and subsequent larval survival on plants. Two rows of maize were planted on one side of a row of established Vetiver grasswhile one row was planted on the other side. The inter-row spacing was 0.75 m with 30 cm between plants and one plant per hill. The Vetiver grass row was one year old and had a dense stand of tillers. Six muslin cloth cages (3.0 x 1.5 m x 1.5 m) were placed transversely over the three rows of maize and one row of Vetiver grass when the maize plants were five weeks old. The maize and Vetiver plants were approximately 0.7 m and 1.0 m high respectively. Each cage formed a replicate and enclosed within it an average of 14 maize plants and a dense 1.2 m long row of Vetiver grass. The surface area inside the cage planted to maize and Vetiver was 2.25 and 0.60 m² respectively. Twenty male and twenty female moths were released into each cage and allowed to oviposit on plants for two nights after which cages were removed. The number of egg batches and eggs per batch on maize was determined by carefully inspecting the foliage of each maize plant. One half of the Vetiver row in each cage was removed from the soil in order to inspect each leaf for egg batches. This was done to facilitate finding of eggs which is difficult in the dense foliage of the grass. There were approximately 54 tillers (splits) of Vetiver in the half-row in each replicate. Leaves were removed from tillers and checked for eggs. The assumption was made that there would be a similar number of eggs on the section of the row that was not removed from the cage. All the maize plants were left in each replicate. Data on mean number of egg batches and mean numbers of eggs on maize and Vetiver was standardized to numbers/m² to facilitate comparison between the two plant species. Data on mean numbers of egg batches, eggs per batch, total number of eggs and larval survival were subjected to Student t-tests to determine if differences were significant.

Results

Moth oviposition preference and larval survival

Laboratory two-choice bioassays

Results from the two-choice experiment with *B. fusca* indicated that there was no significant difference ($t = 0.152$; $P = 0.886$) between the number of egg

batches per plant with 3.3 (S.E. ± 0.66) and 3.0 (S.E. ± 2.08) batches per plant recorded from maize and Vetiver respectively. However, the number of eggs per batch recovered from Vetiver were significantly lower than those on maize (t = 4.176; P = 0.013). Of the total number of *B. fusca* eggs recorded in this experiment 92% was from maize. Results from the first two-choice experiment with *C. partellus* indicated that there were significantly more egg batches per plant on Vetiver (t = –3.528; P = 0.024) (fig.1) while egg batch size did not differ between maize and Vetiver plants (t = –0.231; P = 0.828). The average number of eggs per batch was 33 (S.E. ± 2.2) on maize and 34 (S.E. ± 3.2) on Vetiver. The numbers of eggs per plant was therefore significantly higher on Vetiver (t = 4.294; P = 0.012) with only 18 % of the eggs recorded on maize (fig. 2). The number of *C. partellus* egg batches on rice was significantly higher (t = 3.283; P = 0.030) than on Vetiver. Rice plants received an average of 16 (S.E. ± 3.7) egg batches per plant while maize plants received only 4 (S.E. ± 0.6). The total number of eggs per plant was also significantly lower on Vetiver (t = 3.752; P = 0.019) with 690 (S.E. ± 101.8) and 264 (S.E. ± 50.1) eggs per plant on rice and Vetiver, respectively. However, egg batches were significantly larger on Vetiver (t = 2.893; P = 0.044) with 65 (S.E. ± 3.5) and 44 (S.E. ± 6.1) eggs per batch on Vetiver and rice, respectively. The percentage larval survival of *C. partellus* on potted plants 28 days after infestation (DAI) differed significantly (F = 572.1; P = 0.00001) among plant species (fig. 3). On average 63.0 % and 2.8 % of larvae survived on maize and Napier grass, respectively. No larvae survived on Vetiver grass.

Greenhouse bioassay: Oviposition data

Significantly higher numbers of egg batches (t = 3.932; P = 0.002) were again recorded on Vetiver grass. Only 4.3 % of the total number of egg batches was laid on maize. In this experiment an average of 1.92 (S.E. ± 0.58) egg batches/m² was laid on maize while 155.7 (S.E. ± 37.8) egg batches/m² was laid on Vetiver. The size of egg batches recovered on Vetiver was also significantly larger than those on maize (t = 3.449; P = 0.006), with 34 (S.E. ± 3.8) and 16 (S.E. ± 3.6) eggs per batch for Vetiver and maize respectively. Of the total number of eggs recorded in this experiment 96.3 % were laid on Vetiver which was significantly higher than that on maize plants (t = 2.600; P = 0.026) with 2716.8/m² (S.E. ± 903.6) and 101.3/m² (S.E. ±10.6) being recorded on Vetiver and maize, respectively.

Greenhouse bioassay: Larval survival

The percentage of larval recovery from maize in the greenhouse was significantly higher (t = 6.098;

P = 0.0001) than on Vetiver. The number of larvae recovered 28 days after oviposition was 132 % (S.E. ± 21.6) on maize versus 0.56 % (S.E. ± 0.08) on Vetiver. The recovery rate on maize was therefore 32 % higher than the number of eggs recorded on maize plants.
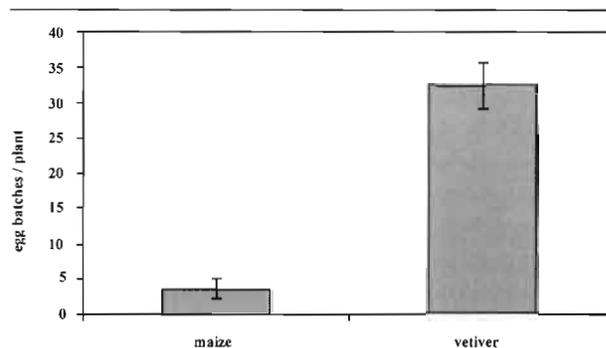

Figure 1
Mean number of egg batches per pot laid by *Chilo partellus* moths on maize and vetiver in two-choice tests. (Bars indicate Standard Error).
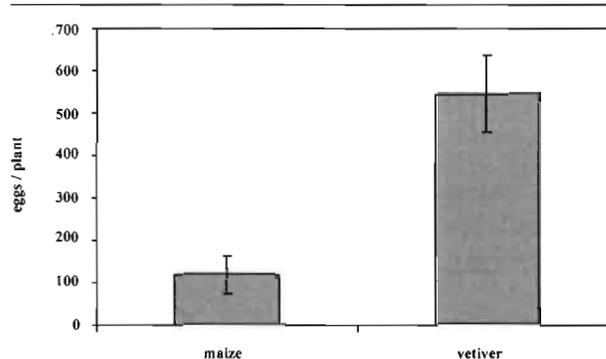

Figure 2
Total number of eggs per pot laid by *Chilo partellus* moths on maize and vetiver in two-choice tests. (Bars indicate Standard Error).
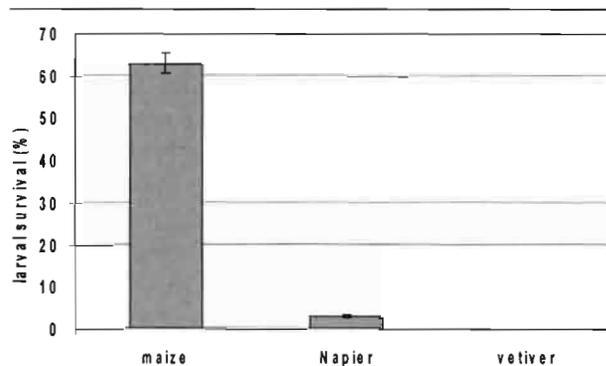

Figure 3
Mean numbers of larvae recovered from maize and vetiver plants in a greenhouse, 28 days after oviposition by *Chilo partellus*. (Bars indicate Standard Error).

Discussion

Busseola fusca moths chose to lay their eggs on maize rather than on Vetiver. The poor status of Vetiver as ovipositional host is ascribed to the physical properties of the plant and *B. fusca* oviposition behaviour. *B. fusca* moths position egg batches between leaf sheaths and the stem of host plants. Since Vetiver "stems" are composed of a large number of tight-fitting leaves with no real stem, no suitable oviposition sites exist. Vetiver grass only produce flower-bearing stems in more tropical areas. Due to the low numbers of eggs recovered on Vetiver no further studies were conducted with *B. fusca*. Both the laboratory and green house experiments showed that *C. partellus* moths chose to lay eggs on Vetiver grass and not on maize. The numbers of larvae recovered from Vetiver plants in all the experiments were low. In the greenhouse experiment more larvae were recovered from maize than the number of eggs actually laid on plants. On maize 132 % of larvae was recovered and on Vetiver grass 0.56 %. The high larval numbers on maize can be ascribed to the unsuitability of Vetiver for first instar larvae and subsequent emigration of larvae from these plants to maize. This is probably due to high levels of larval spin-off and mortality on this plant. After egg hatching many first-instar larvae were observed hanging on silk threads from leaves. The total number of larvae that could have been recovered collectively from maize and Vetiver in the experiment, if 100 % of larvae survived emigration off Vetiver to maize, would have been 5778, but a total of only 986 larvae were recovered from maize. This indicated that in spite of having a suitable host plant (maize) next to the Vetiver plants, larval mortality was still very high with 83 % of larvae not accounted for at the end of the experiment. The high preference and low larval survival of *C. partellus* on Vetiver grass cannot easily be explained. These observations do not support the "preference-performance hypothesis" which states that oviposition preference should correlate with host suitability for offspring development, because females get maximum fitness by ovipositing on the optimal host (Jaenike 1978). It has also been hypothesized that *C. partellus* selects oviposition sites most suitable for egg survival since larvae are mobile and could find a suitable host plant if eggs were laid in close proximity to host plants (van den Berg & van der Westhuizen 1997). Renswick & Chew (1994), in a review of oviposition behaviour in Lepidoptera however observed that larvae are relatively immobile and that the judicious choice of a food plant and the oviposition step is of particular importance in the Lepidoptera. Host specificity may however also play a role in these observed insect/plant interactions. The nature of oviposition choice

in Lepidoptera has been shown to be highly adaptive with females choosing hosts on which larval survival is superior to that on hosts rejected by females (Thompson 1988). Observations on *C. partellus* host selection and offspring performance on sorghum lines with different levels of antibiosis resistance do not support this (van den Berg & van der Westhuizen 1997). The latter authors reported *C. partellus* moth preference for sorghum varieties with high levels of larval antibiosis. If the host plants used in this study for *C. partellus* were put in a preference hierarchy for oviposition it would be rice << Vetiver << maize. Larval survival on rice was not determined but survival rates on Vetiver approached what is referred to by Thompson (1988) as one of the extreme relationships between oviposition preference and offspring performance where females oviposit on a host that is fatal to the immatures. The causes of good or poor performance on a plant species in natural or managed communities does not always result directly from interactions between an insect and plant but could be due to interactions with, amongst others, abiotic differences in microhabitats in which the host plant species grow (Thompson 1988). The relationship between preference and performance can vary under different ecological conditions and is influenced by geographic variation in host use (Thompson 1988). The most feasible explanation for *C. partellus* behaviour on Vetiver in this study is the effect of geography on the growth pattern of the host plant which seldom flowers outside tropical areas. Vetiver produces flowering stems in its area of origin in which *Chilo* sp. is able to survive in very low levels once they enter these stems. This aspect was however not addressed in this study. Low levels of survival of *Chilo* spp. have been reported to infest culms and midribs of leaves wherever Vetiver was planted in Southern China (Xinbao 1992). Field observations made by Shangwen (1999) and Zisong (1991) in China showed high numbers of infested Vetiver plants but low larval recovery. The occurrence of fully grown larvae of another *Chilo* sp., *C. polychysus* (Meyrick), although in low numbers was reported on Vetiver in Vietnam (Truong 2005a & b). *Chilo partellus* is an alien invasive species in Africa. *Chilo partellus* is from the Old World tropics, from where it dispersed to East and Southern Africa during the first half of the twentieth century (Maes 1998). Similarly, Vetiver grass originated in South Asia. India was most probably the primary centre from where it may have dispersed to other areas (Lavania 2000). The strong preference of *C. partellus* for Vetiver grass could therefore possibly be ascribed to an old association between this insect and Vetiver during the period before its current primary host plants (maize, sorghum and rice) were domesticated.

Although this study did not show that *C. partellus* prefers Vetiver to rice the possibility exists that it and also other *Chilo* spp. may choose Vetiver grass to other rice varieties. Many *Chilo* spp., including the notorious *C. partellus* attack rice in many parts of the world (Seshu Reddy 1990). If *Chilo* spp. prefer Vetiver grass it could have a potential as a trap crop around paddy rice fields where it is used as field boundaries and as a soil conservation measure to protect rice from flood damage during the rainy season (Huq 2000).

Vetiver grass has the most important characteristics of a trap crop for *C. partellus*, i.e. it is highly attractive to the target pest (Hokkanen 1991). Once oviposition has taken place, the suitability of the host plant for larval feeding and development is one of the most important aspects of a plant's potential as a trap crop (Hokkanen 1991). Poor larval survival and/or development are also essential for a successful trap crop (Shelton & Nault 2004). If the trap crop has the additional quality that it allows no or very low larval survival, it could be termed a dead-end trap crop (Shelton & Nault 2004). The overall acceptability of the trap plant for feeding is therefore also important (Potting *et al.* 2005) since it prevents the possibility of larval emigration to neighbouring plants, which may be the target food crop, as was observed in the greenhouse study. Infestation levels in this study were however abnormally high and natural enemies that could have a huge impact on survival of migrating larvae were absent from the study.

Conclusions

This study showed that the preference of Lepidopterous stem borer moths to oviposit on certain wild host plants can be exploited in habitat management systems by using these preferred hosts as trap crops in integrated management strategies for stem borers. It also highlighted the phenomenon of lack of correlation between moth preference for host plants and performance of offspring that is sometimes observed in the Lepidoptera. The effect of Vetiver grass as trap crop should be evaluated further and in field experiments under different agro-ecological zones. Furthermore, future studies should be conducted to determined the potential of Vetiver as trap crop for other *Chilo* spp., especially those that attack rice in areas where Vetiver is indigenous.

References

Grimshaw R.G. 2003. *The role of Vetiver grass in sustaining agricultural productivity.* (http://www.vet .org) (accessed on 24/04/2003).

Hokkanen H.M.T. 1991. Trap cropping in pest management. *Annual Review of Entomology* **36**: 119-138.

Huq F. 2000. *Primary and secondary centres of origin of Vetiver and its dispersion.* Proceedings of the Third International Vetiver Conference, Thailand – January 2000. (http://www.vetiver.com/TVN_IVC2/ICV2_index.htm. accessed on 30 April 2003).

Jaenike J. 1978. On optimal oviposition behaviour in phytophagous insects. *Theoretical Population Biology* **14**: 350-356.

Khan Z.R., Chiliswa P., Ampong-Nyarko K., Smart L.E., Polaszek A., Wandera J., Mulaa M.A. 1997. Utilization of wild graminaceous plants for the management of cereal stem borers in Africa. *Insect Science and its Application* **17**: 143-150.

Khan Z.R., Midega C.A.O., Hutter N.J., Wilkins R.M., Wadhams L.J. Assessment of the potential of Napier grass (*Pennisetum purpureum*) varieties as trap plants for management of *Chilo partellus*. *Entomologia Experimentalis et Applicata* (in press).

Lavania U.C. 2000. *Primary and secondary centres of origin of Vetiver and its dispersion.* Proceedings of the Third International Vetiver Conference, Thailand – January 2000 (http://www.vetiver.com/TVN_IVC2/ICV2_index.htm. accessed on 30 April 2003).

Maes K.V.N. 1998. Pyraloidea: Crambidae, Pyralidae, p. 87–98 *in*: Polaszek A. (ed.), *Cereal stem borers in Africa: Economic importance, Taxonomy, Natural enemies and Control.* International Institute of Entomology, CAB International, Wallingford, UK, 530 p.

Midega C.A.O., Khan Z.R., van den Berg J., Ogol C.K.P.O. 2005. Habitat management and its impact on maize stemborer colonisation and crop damage levels in Kenya and South Africa. *African Entomology* **13**: 333-340.

Ndemah R., Gounou S., Schulthess F. 2002. The role of wild grasses in the management of Lepidopterous stem-borers on maize in the humid tropics of western Africa. *Bulletin of Entomological Research* **92**: 507-519.

Potting R.P.J., Perry J.N., Powell W. 2005. Insect behavioural ecology and other factors affecting the control efficacy of agroecosystem diversification strategies. *Ecological Modelling* **182**: 199-216.

Renswick J.A.A., Chew F.S. 1994. Oviposition behavior in Lepidoptera. *Annual Review of Entomology* **39**: 377-400.

Shelton A.M., Nault B.A. 2004. Dead-end trap cropping: a technique to improvemanagement of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Crop Protection* **23**: 497-503.

Seshu Reddy K.V. 1990. Cultural control of *Chilo* spp. in graminaceous crops. *Insect Science and its Application* **11**: 703-712.

Seshu Reddy K.V., Lubega M.C., Sum K.O.S. 1990. Population patterns of *Chilo* spp. in sorghum, maize and millets. *Insect Science and its Application* **11**: 549-554.

Shangwen C. 1999. Insects on Vetiver hedges. *The Vetiver Newsletter* **23**: 17-18.

Singh G., Sandhu G.S. 1978. Oviposition behaviour of maize borer, *Chilo partellus* (Swinhoe) in the field. *Indian Journal of Agriculture* **40**: 191-196.

Thompson J.N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata* **47**: 23-14.

Truong P.N.V. 2005a. Report on the visit to Vietnam, 2-22 January 2005. *Vetiverim* **32**: 2-9.

Truong P.N.V. 2005b. Photographs of the Report on the visit to Vietnam, January 2005. *Vetiverim* **33**: 9.

Van den Berg J. 2006. Oviposition preference and larval survival of *Chilo partellus* (Lepidoptera: Pyralidae) on Napier grass (*Pennisetum purpureum*) trap crops. *International Journal of Pest Management* 52: 39-44.

Van den Berg J., van der Westhuizen M.C. 1997. *Chilo partellus* (Lepidoptera: Pyralidae) moth and larval response to levels of antixenosis and antibiosis in sorghum inbred lines under laboratory conditions. *Bulletin of Entomological Research* 87: 541-545.

Van den Berg J., Rebe M., de Bruyn J., van Hamburg H. 2001. Developing habitat management systems for graminaceous stem borers in South Africa. *Insect Science and its Application* 21: 381-388.

Van den Berg J., De Bruyn A.J.M., van Hamburg H. Oviposition preference andsurvival of the maize stem borer, *Busseola fusca* (Lepidoptera: Noctuidae) on Napier grasses (*Pennisetum* spp.) and maize. *African Entomology*. (in press).

Van Rensburg J.B.J., Walters M.C., Giliomee J.H. 1989. Selective oviposition by the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Journal of the Entomological Society of southern Africa* 52: 105-108.

Xinbao Z. 1992. Vetiver grass in P.R. China. *Vetiver Newsletter* 8: 134-138.

Zisong W. 1991. Excerpts from the experiments and popularization of Vetiver grass, Nanpang Prefecture, Fujian Province, China. *Vetiver Newsletter* 6: 105-108.

Effect of wild grasses planted as border rows on stemborer infestations in maize in Uganda)

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Abstract. The presence of wild grasses in the vicinity of maize fields has been associated with reduced stemborer incidence on the maize crop. This study evaluated the impact of border rows with wild grasses on stemborer infestations and parasitism. Field trials were conducted in East and Central regional Agricultural Research Institutes in Uganda for three seasons. The four grass species planted as border rows included *Pennisetum purpureum* Schumach, *Pennisetum polystachion* (L.) Schult, *Panicum maximum* Jacq. and *Sorghum arundinaceum* (Desv.) Stapf. (Poaceae) were used. A pure maize stand without a grass border was planted as control. *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) were the major stemborers found. Maize with *P. purpureum* and *P. maximum* borders reduced stemborer infestations and damage compared to the pure maize stand. The results were however, not consistent for all the three seasons. Yield per plot was higher in pure maize stand compared to plots with grass borders. The usefulness of this technique in stemborer management is discussed.

Résumé. Effet de graminées sauvages plantées en bordure de champs cultivés sur l'infestation du maïs par les foreurs en Ouganda. On considère souvent que la plantation de graminées sauvages en bordure de parcelles de maïs réduit significativement les dégâts causés par les foreurs. Cette étude évalue l'effet de bordure par des graminées sauvages sur l'action des foreurs ravageurs et sur leur parasitisme. Des essais en champs ont été menés par l'Institut de Recherches Agricoles dans l'Est et l'Ouest de l'Ouganda. Les quatre espèces de graminées sauvages plantées autour des parcelles de maïs ont été: *Pennisetum purpureum* Schumach, *Pennisetum polystachion* (L.) Schult, *Panicum maximum* Jacq. et *Sorghum arundinaceum* (Desv.) Stapf. (Poaceae). Des champs de maïs non bordés de graminées sauvages ont servi de parcelles témoins. Les espèces de foreurs rencontrées en majorité ont été *Busseola fusca* Fuller (Lepidoptera: Noctuidae) et *Chilo partellus* Swinhoe (Lepidoptera: Crambidae). Les parcelles entourées par *P. purpureum* et *P. maximum* ont été plus faiblement infestées par les foreurs que les parcelles témoins. Les résultats n'ont cependant pas été uniformes pour chacune des trois saisons. Le rendement en maïs par champ était plus élevé pour les parcelles témoins que pour celles entourées de graminées sauvages. L'utilisation de ces bordures autour de parcelles cultivées est discutée.

Keywords: Wild host plants, grass borders, stemborers, infestation.

A complex of seven lepidopteran stemborer species is reported to cause economic losses to cereals in Africa. In Uganda, *Busseola fusca* Fuller (Noctuidae) and *Chilo partellus* Swinhoe (Crambidae) are the most important species on maize and sorghum (Ingram 1958; Girling 1978; Matama-Kauma *et al.* 2001). Other species commonly found are *Sesamia calamistis* Hampson (Noctuidae) and *Eldana saccharina* Walker (Pyralidae). All the stemborer species are indigenous to Africa except for *C. partellus*, which was introduced

from Asia into Southern Africa sometime before 1930s (Tams 1932). It was not recorded in East Africa until 1953 (Ingram 1958). Several indigenous parasitoids attack these stem borers but the rates of parasitism are low. *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was recently introduced into Uganda for the control of *C. partellus* and is fully established with parasitism rates of ranging from 4 to 32 % in eastern Uganda (Matama-Kauma *et al.* 2001).

African stemborers have originally attacked only wild grasses and sedges in the tropical and subtropical parts of the continent. With the introduction and cultivation of maize and extensive planting of sorghum, stemborers have followed these cultivated

forms of their host plants (Polaszek & Khan 1998). On wild host plants, stemborer densities do not reach the levels observed in crops, mostly as a result of low survival of young instars (Nye 1960; Mathez 1972; Schulthess *et al.* 1997; Shanower *et al.* 1993; Gounou & Schulthess 2004). Wild grasses have long been considered as reservoirs for stemborers and responsible for pest outbreaks on crops (Ingram 1958; Bowden 1976). However, Schulthess *et al.* (1997) working in Côte d'Ivoire and Cameroon found that the presence of wild hosts in the vicinity of fields reduced stemborer incidence in the crop. They concluded that wild grasses either acted as trap plants or that they stabilized the system for both the pest and natural enemies (Schulthess *et al.* 2001). The presence of wild host plants increases plant diversity and spatial dimensions, which might increase mortality of stemborers as well as be favourable for the conservation of parasitoid by affecting the insects' foraging efficiency in exploiting the host crop. The wild habitat in addition may provide temporal continuity for the natural enemies during the off-season (Overholt *et al.* 1997). Trials that used grasses as trap plants for stemborers have been carried out in Kenya (Khan *et al.* 1997; 2001), Benin and Cameroon (Ndemah *et al.* 2002). The results were variable. Such studies have not been conducted in Uganda. The present study investigated the effect of planting grass border rows on stemborer infestations and parasitism.

Materials and methods

Experimental site

Field trials were conducted at Namulonge Agricultural Research Institute (NAARI; latitude 0°31'N, longitude 32°36'E) and Serere Agricultural Research Institute (SAARI; latitude 1°35'N, longitude 33°31'E) in central and eastern Uganda, respectively. Both sites are characterised by a bimodal rainfall distribution, which allows for two cropping seasons, the first lasting from March to mid-July and the second from September to December. The vegetation at Serere is savannah grassland with a long dry spell between December and February while Namulonge is characterised by a forest savannah mosaic.

Experimental procedures and layout

The trials were conducted during three rainy seasons, from May to September (first rainy season), September 2004 to January of 2005 (second rainy season), and April to August of 2005. The grass species used in the study included *Pennisetum purpureum* Schumach, *Pennisetum polystachion* (L.) Schult, *Panicum maximum* Jacq. and *Sorghum arundinaceum* (Desv.) Stapf (Poaceae). They were selected based on a survey of abundance of stem borers on wild grasses conducted in 2003. These grasses were found in relatively high abundance close to maize fields and with stemborer damage (Matama-Kauma *in lit.*). Each of the four grasses was planted as border rows surrounding maize plots. The trials were laid in a randomised complete block design with split plots and replicated three times. The main plot treatments consisted of maize surrounded by grass border of one of the species and a pure maize stand as control. Plot size was 14 x 20 m in the pure maize stand, and 11 x 17 m in the maize-grass border treatment, and each plot was divided into subplots of 14 x 10 m and 11 x 9 m, respectively, which were treated with insecticide and left untreated. The distance between blocks was 5 m and that between main plots was 3 m while subplots were separated by 1 m. The open pollinated 105-days maize variety Longe 4 was planted at the spacing of 75 x 50 cm with three seeds per hill. It was thinned down to two plants at 2 weeks after plant emergence (WAE). The grass borders were established as three rows of grass tufts planted at the same spacing two weeks before maize was planted. In the insecticide treatment subplot, Furadan granules (i.e carbofuran 5G, 50 g/kg a.i) were applied into the whorls at 3WAE.

Data collection

Each sub plot was divided into four quadrants. At three WAE, 10 plants of maize were randomly uprooted per quadrant and assessed for stemborer egg batches. The eggs found were collected, counted and kept in the laboratory until larvae or parasitoids emergence. A second sampling was conducted at maize tasselling stage to assess stemborer infestation and larval parasitism. Five plants were randomly selected per quadrant and assessed for infestation. Plants infested and the number of larvae and pupae per plant were recorded. All the larvae collected were reared in the laboratory on maize stems and cobs which were changed every 2–3 days until pupation or parasitoids emergence. The pupae obtained from the field were singly placed in Petri dishes and kept until parasitoid or adult moth emergence. Parasitoids that emerged were preserved in 70 % alcohol and identified where possible. Those not identified were sent to the International Centre of Insect Physiology and Ecology (ICIPE) in Kenya for further identification.

At maturity, plant height with tassel, tunnel length, number of borers and cob weight were measured on five maize plants per quadrant. All maize cobs of plants from 2 m² of the two middle rows in each quadrant were harvested and weighed. The sampling procedures were the same for all seasons and sites except for the first season 2004 when there was no insecticide treatment. Percentage stem tunnel was calculated as the ratio of stem tunnel divided by the plant height.

Statistical analyses

The data were analysed using SAS software (SAS 2001). Analysis of variance (ANOVA) was used to assess treatment differences between wild grass borders and insecticide effect in stemborer infestation, plant damage and yield variables. The data were analysed separately for each season and site. Where necessary, the data that involved insect counts were $\log(x + 1)$ transformed and proportions were arcsine square root transformed before analyses. The effect of grassy borders on parasitism was evaluated using a log-likelihood test in a two dimensional contingency table (Zar 1999). The significance level was set at $P < 0.05$ and means were compared with Students-Newman-Keuls multiple range test (SNK). Back transformed means are presented.

Results

Stemborer infestation and parasitism

Four stemborer species namely: *C. partellus*, *B. fusca*, *S. calamistis* and *E. saccharina* were recovered. Egg infestation was very low with 0–5 % of the plants being infested and there were no significant differences

across grass border treatments (data not shown). *Chilo partellus* and *B. fusca* were the dominant species accounting for over 90 % of all the species in both sites (figs. 1 & 2). Stemborer infestations on maize varied across sites and seasons. There were no significant differences between wild grass border treatments in the first season, when grasses had not fully established at both sites (figs. 1a & 2a). Changes in percentage of infested plants had a similar tendency as the density of total stemborers in the following seasons. At Namulonge, *C. partellus* and *B. fusca* occurred in equal proportions except for the second rains of 2004 when *C. partellus* became the most abundant species (fig. 1). The total stemborer density decreased with seasons in plots with maize only, maize with *P. purpureum* and *S. arudinaceum* border (fig. 1). Percentage of infested plants and *C. partellus* density was significantly reduced in maize with *P. purpureum* grass borders in the second and third seasons (fig. 1b & c). *Chilo partellus* was

the dominant species, representing 70–100 % of the stem borer populations at Serere, with the exception of the first rains of 2004 (fig. 2). Stemborer densities were low during both seasons of 2004 and increased dramatically in the third season (first rains 2005). In this season, maize with grass border rows significantly reduced the number of stemborers per plant compared with pure maize although *P. purpureum* was the only grass border that significantly reduced the number of infested plants (fig. 2b & c).

In this study, *B. fusca* eggs were found parasitized by *Telenomous busseolae* Gahan (Hymenoptera: Scelionidae). Larval parasitism was mostly due to the braconids *Cotesia sesamiae* Cameron and *C. flavipes* and ranged between 3 and 33 %. A Chi-square test showed that at Namulonge the proportion of parasitized larvae by the two *Cotesia* spp was dependent on grass border treatments (tab. 1). Higher levels of parasitism were observed in maize plots with *P. maximum* and
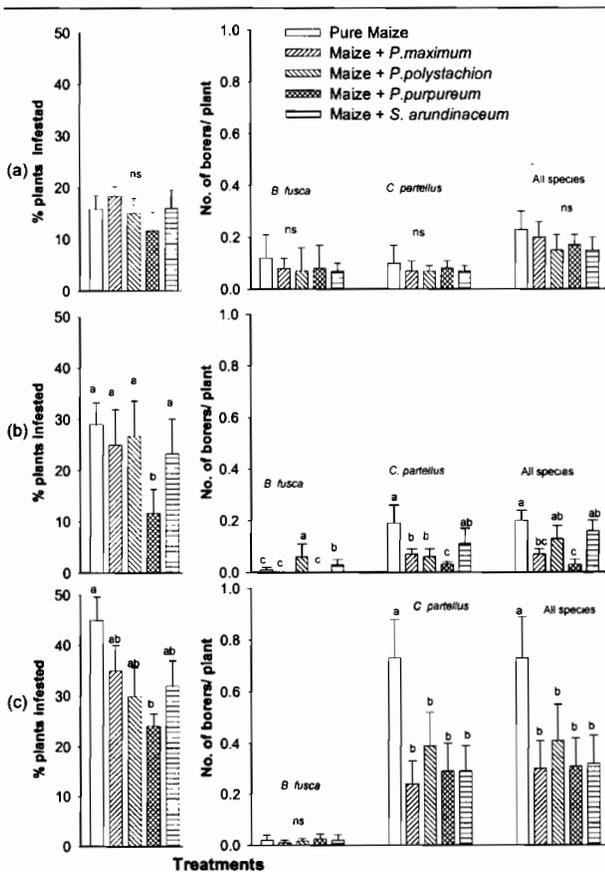

Figure 1
Percentage plants infested and number of borers per plant in pure maize and maize with grassy borders in the first (a) and second rains (b) of 2004, and first rains 2005 (c) at Namulonge. Columns followed by the same lower case letter were not significantly different at P ≤ 0.05.
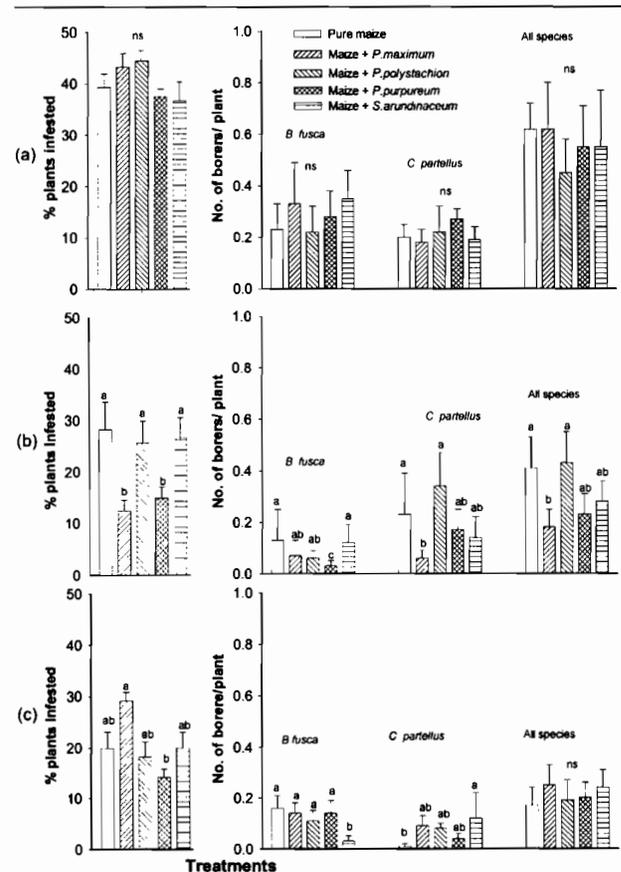

Figure 2
Percentage plants infested and borer density in pure maize and maize with grassy borders in the first (a) and second rains (b) of 2004, and first rains of 2005 (c) at Serere. Columns followed by the same lower case letter were not significantly different at P ≤ 0.05.

P. polystachion borders while *P. purpureum* had the lowest level of parasitism. At Serere, however, there were no significant differences in proportion of larvae parasitized across treatments. Pupal parasitoids found included *Dentichiasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae) on *C. partellus* pupae and *Pediobus furvus* Gahan (Hymenoptera: Eulophidae) on both *C. partellus* and *B. fusca* pupae.

Plant damage and yield

The percentage of tunnelled stems in maize was not significantly different across treatments at both Namulonge and Serere except for the first rains of 2005 at Serere where it was higher in pure maize than in maize with grass borders (tab. 2). Insecticide treatment reduced the percentage of tunnelled stems at Namulonge in one of the two seasons, while at Serere the results were variable. Insecticide treated maize with *P. maximum* border rows had significantly increased cob weight than pure maize stands during the first rainy season of 2005 at Namulonge. At Serere untreated maize plots surrounded by *P. maximum* border rows had a significantly higher cob weight than pure maize and maize surrounded by other grassy borders (tab. 2). Application of insecticide did not have a significant effect on maize yield except for a few instances at both sites. Maize yield per plot was significantly higher in pure maize stands than in maize with grass border rows except for the first rainy season of 2005 at Namulonge and first rainy season of 2004 at Serere.

Discussion

At both sites, grass border rows around maize fields showed no consistent effect on *B. fusca* and *C. partellus* infestations and on yields of maize. In western Africa, incidence of stemborers such as *B. fusca*, *S. calamistis* and *E. saccharina* in maize crops decreased with the abundance of grasses around the field (Gounou *et al.* 1994; Schulthess *et al.* 1997). In subsequent trials in the humid forest of Cameroon in 1996 and 1997 by Ndemah *et al.* (2002), border rows with grasses reduced pest densities in maize, in the second and third season after planting the borders, but not during the first season. In this study, grass borders did not show significant effects on stemborer infestations in the first season indicating that the borders have to be well-established to have an effect. In similar trials in the forest-savannah

mosaic of Benin in 1998 and 99, one set of trials showed a decrease in pest densities, while another set showed no effect (Ndemah *et al.* 2002). Additionally, in the Cameroon trials during the second cropping season of 1997, when there was a prolonged drought, Ndemah *et al.* (2002) observed less wilting and more vigorous plants in crops surrounded by *P. purpureum*. Thus, they hypothesized that the main effect of grass border rows was due to changes of the soil chemical and physical properties in plots surrounded by the tall grasses rather than to lower pest densities. Follow-up trials in 2002 indicated a higher soil water retention capacity in plots surrounded by grasses compared to plots with only maize. It was concluded that grass border rows had only a beneficial effect if the crop was affected by spells of drought, which is crucial during tasseling of maize (Ndemah *et al. in lit.*).

Ndemah *et al.* (2002) showed that the presence of wild grasses increased egg and larval parasitism in maize. Again, follow-up trials in 2002 showed no effect on parasitism, thus corroborating results of the present experiments where some of the grass borders had a significant effect on larval parasitism at only one of the two sites. In Kenya, Midega *et al.* (2005) observed that agro-forestry and cowpea intercropping systems compared to maize monocrop did not influence the effectiveness of the larval parasitoids, *C. sesamiae* and *C. flavipes*.

What are the reasons for the variability in the effects of grass border rows? First, borer species compositions vary greatly with regions in Africa. The major species in West Africa, *S. calamistis* and *E. saccharina* do not diapause and are therefore forced to spend the off-season on wild hosts. Consequently they never develop an oviposition preference for maize, which guarantees a much higher survival of offspring than wild grasses (Shanower *et al.* 1993). This was corroborated by oviposition preference studies by Schulthess *et al.* (1997). The stemborers *B. fusca* and *C. partellus*, on the other hand, do diapause during the off-season thus they do not depend on alternative wild hosts for perennation of their populations.

The host plant range of *B. fusca* and *C. partellus* is probably much narrower than that of *S. calamistis* and *E. saccharina* (Gounou & Schulthess 2004; LeRü *et al. in lit.*) because they undergo diapause. This suggests that grass border rows would be more effective against

Table 1. Proportion of larvae parasitized by *Cotesia flavipes* and *C. sesamiae* in pure maize and maize with grass borders at Namulonge and Serere*.

Site	Pure maize	Maize + Pm	Maize + Ppo	Maize + Ppu	Maize + Sar	Chi^2	P
Namulonge	14.8 (8)	20.6 (7)	18.9 (10)	3.3 (1)	10.9 (5)	19.09	0.0008
Serere	11.8 (4)	9.1 (4)	8.3 (2)	14.8 (4)	14.3 (5)	3.13	0.54

* Pm = P. maximum; Ppo = P. polystachion; Ppu = P. purpureum; Saru = S. arundinaceum; numbers of parasitized larvae in parentheses.

non-diapausing than diapausing species. In fact, in studies by Ndemah *et al.* (2002), the diversity of borers and parasitoids and differences in parasitism between treatments were greater in the Benin trials, where *S. calamistis* and *E. saccharina* were the main species, than in the Cameroon trials, where *B. fusca* was the predominant pest. In the present study the populations of *S. calamistis* and *E. saccharina* were very low and hence the effect of grass borders on these species could not be measured.

The question also arises if grasses act as trap plants for diapausing species such as *B. fusca* and *C. partellus* or if they rather form a barrier. This could explain the variable results of the border rows. If the borders are not well established, the moths have easy access to and oviposit on the crop. Moreover, if crop residues are not removed, they form a refuge for diapausing larvae. The adults emerging at the onset of the season will be arrested

inside the border rows and attack the new crop rather than disperse. The barrier hypothesis is partly supported by findings by Randriamananoro (1996), Calatayud *et al.* (*in lit.*) and van den Berg *et al.* (*in lit.*) who found that ovipositing *B. fusca* did not prefer *P. purpureum* over maize. *Pennisetum purpureum* is used as 'pull' in the "push-pull" strategy but Nye (1960) citing Wilkinson (1936) already indicated that *B. fusca* does not oviposit on this grass species. As shown by Le Rü *et al.* (*in lit.*), *B. fusca* and *C. partellus* attack very few wild host plant species, with over 90 % found on wild sorghum. Le Rü *et al.* (*in lit.*) suggest that borers on wild host plants have been widely misidentified. Similarly, recent surveys in Cameroon found that *B. fusca* was exceedingly rare on wild grasses including *P. purpureum*, where the most common species were *Poenoma serrata* Hampson (Lepidoptera: Noctuidae) and *Sesamia* sp.

Table 2. Effect of grass border rows and the insecticide treatment interaction on means (± SE) of % stem tunnelled, cob weight and cob weight per plot in the first and second rains 2004 and first rains 2005 at Namulonge and Serere[1].

Grass border	Namulonge[2]				1st rains 2004	Serere			
	2nd rains 2004		1st rains 2005			2nd rains 2004		1st rains 2005	
	Untreated	Treated	Untreated	Treated	Untreated	Untreated	Treated	Untreated	Treated
% stem tunnel									
Pure Maize	0.7 ± 0.2	0.4 ± 0.17	2.0 ± 0.5A	0.4 ± 0.2B	0.4 ± 0.42	3.0 ± 0.8	2.4 ± 0.9	4.6 ± 1.1aA	0.9 ± 0.4B
*Maize + Pm**	0.8 ± 0.3	0.2 ± 0.17	2.5 ± 0.7A	0.7 ± 0.2B	1.4 ± 0.82	3.5 ± 0.8A	0.5 ± 0.4B	2.5 ± 0.6b	1.3 ± 0.6
Maize + Ppo	1.1 ± 0.4	0.4 ± 0.17	1.5 ± 0.7A	0.1 ± 0.1B	0.8 ± 0.63	2.6 ± 1.0	1.1 ± 0.7	1.8 ± 0.5b	2.3 ± 0.7
Maize + Ppu	0.6 ± 0.3	0.3 ± 0.14	1.9 ± 0.8A	0.4 ± 0.3B	1.0 ± 0.7	1.5 ± 0.5	0.7 ± 0.4	1.3 ± 0.4b	1.1 ± 0.4
Maize + Saru	1.8 ± 0.6	0.8 ± 0.32	1.4 ± 0.6	0.9 ±0.4	1.4 ± 0.8	2.4 ± 0.7	1.1 ± 0.5	1.5 ± 0.5b	2.3 ± 1.0
df	4, 295	4, 295	4, 274	4, 263	4, 275	4, 246	4, 246	4, 244	4, 196
F-value	1.01	0.94	0.91	0.97	1.45	0.86	0.49	3.13	1.06
P	0.4028	0.4404	0.4558	0.4232	0.2180	0.4882	0.2046	0.0156	0.376
Cob weight/ plant (g)									
Pure Maize	171.3 ± 6.9	179.9 ± 7.1	143.2 ± 9.9	132.5 ± 7.4b	147.9 ± 9.0	81.0 ± 5.1ab	71.2 ± 4.6	116.1 ± 6.5ab	127.9 ± 4.6
Maize + Pm	166.2 ± 8.3A	196.9 ± 8.4B	161.3 ± 9.9	176.3 ± 9.0a	141.6 ± 9.2	91.9 ± 4.8aA	69.0 ± 3.9B	122.8 ± 6.3a	122.1 ± 5.6
Maize + Ppo	162.0 ± 9.1	177.5 ± 8.9	129.4 ± 8.2A	168.3 ± 8.4aB	144.4 ± 7.6	67.6 ± 4.2b	60.9 ± 4.5	122.8 ± 7.8a	111.9 ± 5.7
Maize + Ppu	155.2 ± 8.4	166.4 ± 7.9	141.6 ± 9.7	158.3 ± 8.9ab	151.7 ± 7.6	72.9 ± 4.2b	73.3 ± 4.2	114.9 ± 6.4ab	112.7 ± 5.6
Maize + Saru	173.0 ± 8.4	172.6 ± 8.4	145.2 ± 8.6	148.8 ± 8.3ab	150.9 ±9.2	72.5 ± 4.3b	77.6 ± 4.6	92.5 ± 7.2bA	121.9 ± 11.5B
df	4, 295	4, 295	4, 274	4, 263	4, 275	2, 246	4, 246	4, 244	4, 196
F-value	1.06	2.37	1.64	4.21	0.25	4.26	1.84	2.64	1.22
P	0.3777	0.0526	0.1652	0.026	0.9110	0.0024	0.1219	0.035	0.3047
Cob weight/plot (kg)									
Pure Maize	221.9 ± 3.2aA	241.3 ± 6.0aB	118.0 ± 7.3	117.6 ± 5.9a	97.5 ± 19.5	118 ± 5.5a	115.4 ± 8.8a	134.1 ± 13.2a	147.8 ± 10.8a
Maize + Pm	131.3 ± 5.4bA	165.6 ± 5.4bB	112.7 ± 6.4	112.7 ± 6.6a	80.9 ± 15.0	79.2 ± 6.5b	61.0 ± 5.6 b	83.2 ± 8.1b	78.6 ± 6.1b
Maize + Ppo	137.6 ± 7.8b	143.2 ± 5.5b	100.5 ± 5.8	114.2 ± 7.7a	67.8 ± 6.5	50.2 ± 6.2b	50.5 ± 3.6b	84.3 ± 9.4b	75.4 ± 6.4b
Maize + Ppu	131.9 ± 8.1b	146.2 ± 13.8b	97.4 ± 7.7	104.2 ± 4.1ab	71.0 ± 13.8	53.1 ± 8.7b	70.6 ± 11.1b	78.3 ± 6.9b	74.3 ± 5.7b
Maize + Saru	146.7 ± 4.0b	145.1 ± 10.9b	94.3 ± 7.4	87.2 ± 6.8b	64.4 ± 11.8	65.0 ± 8.9b	70.1 ± 11.8b	64.6 ± 1.4b	80.9 ± 6.8b
df	4, 10	4, 10	4, 55	4, 55	4, 10	4, 10	4, 10	4, 21	4, 21
F-value	40.66	21.52	2.17	3.70	0.92	8.00	14.42	7.52	16.97
P	<.0001	<.0001	0.0847	0.0097	0.4900	0.0037	0.0004	0.0006	0.0001

Within a season, means within rows followed by the same uppercase letters and means within columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (SNK); [1]Pm = *P. maximum*; Ppo = *P. polystachion*; Ppu = *P. purpureum*; Saru = *S. arundinaceum* border; [2]Yield data was not collected at Namulonge during the first rains 2004 due to drough.

Concerning *C. partellus*, why should an insect species oviposit on a plant species that causes a 100 % offspring mortality? According to Singer *et al.* (1988) and Ng (1988) oviposition preference and larval performance may be correlated such that females prefer the plant species on which their larvae have the greatest chance of surviving during their first 10 days of growth. Two reasons are proposed for the preference of an unsuitable host plant by *C. partellus*: a) *C. partellus* is exotic to Africa and *P. purpureum* is a relatively recent addition to its habitat, b) *C. partellus* does not have a preference for *P. purpureum* over maize. Field evidence, which would consist of higher number of eggs laid on *P. purpureum* compared to maize is lacking. Existing data do not thus support either of the hypotheses.

The main reason for the variable results of the technology, however, might be that the area planted with grasses is too small to have an effect. The surveys carried out in Côte d'Ivoire and Cameroon showed that borer densities on maize steeply decreased with grass abundance around the field. These grass habitats very likely have been well established for many years, therefore, harbouring stable populations of both pests and parasitoids. With the exception of the present study and experiments in the humid forest zone in Cameroon (Ndemah *et al.* 2002; 2003), the abundance of wild grasses in the vicinity of the trials has not been assessed. Those grass habitats might have had a much more crucial effect on the population dynamics of pests and beneficials than the grass border rows. From the foregoing it can be concluded that the role of grass border rows in controlling cereal stemborers in crops is questionable and that, moreover, the mechanisms are not understood. As proposed by Ndemah *et al.* (2002), leaving wild habitats in the vicinity of crop fields intact rather than burning them every dry season might have more effect on pest populations in crops than planting grass border rows.

References

Bowden J. 1976. Stem borer ecology and strategy for control. *Annals of Applied Biology* 84: 107-111.

Girling D.J. 1978. The distribution and biology of *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) and its relationship with stem-borers in Uganda. *Bulletin of Entomological Research* 68: 411-488.

Gounou S., Schulthess F., Shanower T., Hammond, W. H. O., Braima H., Cudjoe A.R., Adklaoe R., Antwi K. K., Olaleye I. 1994. *Stem and ear borers of maize in Ghana.* Plant Health Management Research Monograph 4, International Institute of Tropical Agriculture, Ibadan, Nigeria, 23 p.

Gounou S., Schulthess F. 2004. Spatial distribution of lepidopterous stemborers on wild host plants in West Africa and its implications for sampling schemes. *African Entomology* 12: 172-177.

Ingram W.R. 1958. The Lepidopterous stalk borers associated with Graminae in Uganda. *Bulletin of Entomological Research* 49: 367-383.

Khan Z.R., Chiliswa P., Ampong-Nyarko K., Smart L.E., Polaszek A., Wandera J., Mulaa M.A. 1997. Utilisation of wild gramineous plants for the management of cereal stemborers in Africa. *Insect Science and its Application* 17: 143-150.

Khan Z.R., Pickett J.A., Wadhams L.J., Muyekho F. 2001. Habitat management strategies for the control of cereal stemborers and striga in maize in Kenya. *Insect Science and its Application* 21: 375-380.

Matama-Kauma T., Kyamanywa S., Ogwang J.A., Omwega C.O., Willson H.R. 2001. Cereal stemborer species complex and establishment of *Cotesia flavipes* Cameron in eastern Uganda. *Insect Science and its Application* 21: 317-326.

Mathez F.C. 1972. *Chilo partellus* Swinhoe, *C. orichalcociliellus* Strand (Lepidoptera: Crambidae) and *Sesamia calamistis* Hmps. (Lepidoptera: Noctuidae) on maize in the Coastal Province, Kenya. *Mitteilungen Schweizerischen Entmoologischen Gesellschaft* 45: 267-289.

Midega A.C.O, Ogol C.K.P.O. Overholt W.A. 2005. Life tables, key factor analysis and density relations ro natural populations of the spotted stemborer, *Chilo partellus* (Lepidoptera: Crambidae) under different cropping systems at the Kenyan coast. *International Journal of Tropical Insect Science* 25: 86-95.

Ndemah R., Gounou S., Schulthess F. 2002. The role of wild grasses in the management of lepidopterous cereal stemborers on maize in western Africa. *Bulletin of Entomological Research* 92: 507-519.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Poehling M., Cardwell K.F. 2003. Factors affecting infestations of the stalk borer *Busseola fusca* (Fuller) on maize in the forest Zone of Cameroon with special reference to scelionid egg parasitoids. *Environmental Entomology* 32: 61-70.

Ng D. 1988. A novel level of interactions in plant-insect systems. *Nature* 334: 611-13.

Nye I.W.R. 1960. The insect pests of graminaceous crops in East Africa. *Colonial Research Studies* 31: 1-48.

Overholt W.A., Ngi-song A.J., Omwega C.O., Kimani-Njogu S.K., Mbapila J. C., Sallam M. N., Ofomata V. 1997. A review of the introduction and establishment of *Cotesia flavipes* Cameron in East Africa for biological control of cereal stemborers. *Insect Science and its Application* 17: 79-88.

Polaszek A., Khan Z.R. 1998. Host Plants, p. 3-10 *in*: A. Polaszek (ed.), *African cereal stemborers: Economic importance, taxonomy, natural enemies and control.* CAB International 1998, University Press, Cambridge, UK.

Randriamananoro J.J. 1996. *Behavioural and physiological responses of the cereal stalk borer Busseola fusca Fuller (Lepidoptera: Noctuidae) to selected cultivated and wild host plants.* PhD thesis, Rivers State University of Sciences and Technology, Port Harcout, Nigeria, 241 p.

SAS Institute 2001. *Users' Guide: Statistics, release 8.2* SAS Institute Inc., Carry, North Carolina, USA.

Schulthess F., Bosque-Perez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Goergen G. 1997. Exchange of natural enemies of lepidopteran cereal stemborers between African regions. *Insect Science and its Application* 17: 97-108.

Schulthess F., Chabi-Olaye A., Georgen G. 2001. Seasonal fluctuation of *Sesamia calamistis* (Lepidoptera: Noctiudae) populations in maize fields in southern Benin. *Biocontrol Science and Technology* 11: 756-777.

Shanower T., Schulthess F., Bosque-Pérez N.A. 1993. The effect of larval diet on the growth and development of *Sesamia calamistis* Hampson (Lepidoptera: Noctiudae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Insect Science and its Application* 14: 681-685.

Singer M.C., Ng D., Thomas C.D. 1988. Heritability of oviposition preference and its relationship to offspring performance within a single insect population. *Evolution* 42: 977-85.

Tams W.H.T. 1932. New species of African Heterocera. *Entomologist* 655: 1241-1249.

Wilkinson H. 1936. *Report of the Entomological section.* Representative 2, Department of Agriculture, Kenya 1935, p. 60-70.

Zar J.H. 1999. *Biostatistical Analysis*, fourth ed. Prentice-Hall, Upper Saddle River, New Jersey 663 p.

The effect of grassy field margins and fertilizer on soil water, plant nutrient levels, stem borer attacks and yield of maize in the humid forest zone of Cameroon

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Abstract. Two field experiments, planted in a split plot design, were conducted during 2002 in the forest zone of Cameroon, to investigate the effect of border rows with *Pennisetum purpureum* (Poaceae) or with *Panicum maximum* (Poaceae) on soil water, plant nitrogen (N), phosphorus (P) and potassium (K), borer infestations, parasitism and maize yield. The grassy boundaries were the main plots and fertilizer treatment the sub plots. Soil humidity was significantly higher under the grass borders than in maize plots. Nitrogen uptake by maize tended to be highest in plots surrounded by *P. purpureum* but the differences were significant during the second season only. *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) was the predominant borer species followed by *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae). The predominant parasitoid species was the scelionid egg parasitoid *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae). During both seasons, plant nitrogen, *B. fusca* infestation, plant damage and yield were significantly higher in maize sub-plots that received fertilizer The interaction between the grassy margin effect and the fertilization was significant only for *B. fusca* infestations, during the second season with maize + *P. purpureum* having a significantly lower number of borers in the fertilized than the unfertilized sub-plots. The grassy borders had no effect on *B. fusca* egg parasitism variables except in the first season, when maize with *P. purpureum* had a significantly higher percentage of egg batches parasitized. During both seasons, there were some significant differences in yield variables between main plots but the trends were not clear. Multiple regression showed that *B. fusca* infestation, plant damage, egg parasitism, plant N, P and K affected yield, with plant nutrients explaining most of the variability. The implication of the findings for the feasibilty of this habitat management technology to farmers in southern Cameroon is discussed.

Résumé. L'effet de bordure herbeuse et de la fertilisation sur la teneur en eau des sols, les nutriments de la plante, l'infestation par les foreurs et le rendement du maïs dans la zone de forêt humide du Cameroun. Deux essais au champ, dans des parcelles subdivisées ont été menés en 2002 dans la zone de forêt du Cameroun, pour étudier l'effet de bordure de *Pennisetum purpureum* (Poaceae) ou *Panicum maximum* (Poaceae), sur l'humidité du sol, les teneurs en azote, phosphore et potassium de la plante, l'infestation par les foreurs des tiges, leur parasitisme et le rendement du maïs. Les bordures herbeuses étaient les parcelles principalement à l'étude avec l'utilisation d'engrais pour les sous-parcelles de contrôle. L'humidité du sol était significativement plus élevée en bordure qu'à l'intérieur des parcelles de maïs. La prise en azote par le maïs avait tendance à être plus élevée dans les parcelles entourées par *P. purpureum* mais ceci nê fût observé que dans la deuxième campagne d'évaluation. *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae) a été le foreur le plus recensé suivi par *Eldana saccharina* (Walker) (Lepidoptera : Pyralidae). Le parasitoïde le plus abondant était *Telenomus busseolae* (Gahan) (Hymenoptera : Scelionidae). Pendant les deux campagnes d'évaluation la teneur azotée, l'infestation par *B. fusca*, les dégâts et le rendement étaient significativement plus élevés dans les sous-parcelles qui ont reçu de l'engrais. L'interaction entre l'effet de bordure et la fertilisation par engrais n'était significative que pour le paramètre « infestation par *B. fusca* » dans la deuxième campagne avec maïs + *P. purpureum* ayant le moins de ravageur dans les sous-parcelles fertilisées que dans celles non-fertilisées. Les bordures herbeuses n'ont pas eu d'effet sur le parasitisme des œufs de *B. fusca*, sauf pendant la première campagne d'évaluation où le maïs + *P. purpureum* avait significativement plus de masses d'œufs parasitées. Pendant les deux campagnes, il y avait quelques différences dans le rendement selon le traitement mais aucune tendance marquée fût observée. Des régressions multiples ont permis de montrer que l'infestation par *B. fusca*, les dégâts de la plante, le parasitisme des œufs et les teneurs en azote, potassium et phosphore du maïs ont affecté le rendement, les nutriments de la plante expliquant les variabilités. Les conséquences de ces résultats sur l'utilisation d'une telle technique culturale dans le milieu paysan en zone forestière sont discutées.

Keywords: *Busseola fusca*, stem borer, grasses, soils, Africa.

The most cited constraints of field grown maize in Central Africa are poor soil fertility (Mokwunye & Vlek 1986; Hauser & Nolte 2002; Hauser et al. 2002; Nolte et al. in lit.) and lepidopterous stem borers (Cardwell et al. 1997; Bosque-Pérez & Schulthess 1998; Ndemah et al. 2001a; Ndemah & Schulthess 2002). Two borer species, namely the noctuid *Busseola fusca* (Fuller 1901) and the pyralid *Eldana saccharina* Walker 1865, cause yield losses in both quantity and quality ranging from 10–70%.

Recently, emphasis is being given in Cameroon to research on habitat management as part of Integrated Pest Management technologies to control maize stem borers. Increasing plant biodiversity via mixed cropping and increasing soil fertility via rotation of maize with leguminous cover and grain crops was shown to result in a considerable reduction in yield losses caused by borers (Chabi-Olaye et al. 2005a & b). Mechanisms involved were lower pest density in intercrops as a result of reduced host finding by the ovipositing female moth, and enhanced ability of plants to compensate for pest damage, if soil fertility was increased. By contrast, in East Africa, major emphasis is given to using wild plant hosts of stem borers, grasses mainly, as trap plants to reduce borer infestations on crops (Khan et al. 1997; 2001; van den Berg et al. 2001). Similar work by Ndemah et al. (2002) in the derived savannah of Benin and in the humid forest zone of Cameroon showed that planting grassy border rows considerably reduced stem borer infestations on maize and in some cases doubled yields. In Benin, grassy border rows considerably increased egg and larval parasitism on maize while in Cameroon the lower pest densities were attributed to the border rows acting as trap plants mainly. In addition, it was speculated that the tall *Pennisetum purpureum* Moench (Poaceae), used as border rows in Cameroon, might have had a beneficial effect on soil chemical and physical properties by reducing soil erosion or increasing soil water holding capacity, leading to higher yields.

The objectives of this work were to assess the effect of border rows with *P. purpureum* and *Panicum maximum* Jacq. (Poaceae) on soil water, plant N, P and K, stem borer infestations and natural enemy activities as well as yields, in a forest margin site in Cameroon.

Materials and Methods

Experimental site

The work was carried out at Nkometou 111 Essong in the humid forest zone of southern Cameroon at latitude 4°05'N and longitude 11°33'E. The rainfall pattern is bimodal with a long first rainy season from mid-March to mid-July and a short second season from mid-August to end of November. There is a short unreliable dry spell, the August break, separating the two seasons. Average annual rainfall is around 1500 mm. The experiment was planted on an oxisol with clayey sand in the top 30 cm layer, consisting of 51.8% sand, 35.2% clay and 13.0% silt. The chemical composition before the experiments were planted was: pH (water) 5.1, total % C, 2.6%, cmol/kg of Ca 4.03, Mg 1.54 and Al 0.03, total % N of 0.16, 10.8 ppm of P and 0.19 meq of K.

Experimental procedures

Two field trials were conducted from March to August and from September to December 2002. The experimental design was a split plot with three main and two sub-plot treatments arranged in four blocks (i.e., four replicates) planted each in a different farmer's field. During the first three weeks of March 2002, either tufts of *P. maximum* or 50 cm-long young stem pieces of *P. purpureum* were planted in a 2-meter wide perimeter around two randomly chosen plots of 22 x 22 m, in each block; the grass-less control plot was 20 m x 20 m. The two species were chosen because in earlier experiments they have been shown to reduce densities of noctuid stem borers and increase parasitism in adjacent maize crops (Ndemah et al. 2002). The plots were seperated by 3 m. The *P. maximum* tufts were spaced at 50 and 25 cm between and within rows, respectively, while the stem pieces of elephant grass were spaced at 50 cm between and placed end-to-end within rows, making a total of five rows per grass species. The treatments will be referred to as maize only (control), maize + Pm (maize surrounded by *P. maximum*) and maize + Pp (maize surrounded by *P. purpureum*).

In the third week of April, in each plot, a 20 m x 20 m area was planted with the open-pollinated 120-days maize variety Cameroon maize series CMS 8704. The distance between maize and the grassy border row was 1 m. Maize was spaced at a distance of 75 cm and 50 cm between and within rows, respectively. Four seeds were planted per hill and the crop thinned down to two plants per pocket, 14 days after planting (DAP). The maize plots were divided into two 10 m x 20 m sub-plots. The compound NPK fertilizer (20-10-10) was applied as side dressing at the rate of 200 kg per ha in one of the sub-plots, chosen at random, seven DAP. Two hand-weedings were done at 28 and 56 DAP. At 28 DAP, urea fertilizer (46%), at the rate of 92 kg of N per ha was applied as side dressing in the same sub-plots that had received NPK.

In the second season, during the second week of September 2002, another maize crop was planted in the same plots with the same treatment and agronomic practices applied as in the first season.

Data collection

Stemborers and parasitoids

At 42 DAP, each maize sub-plot was divided into four quadrats of five by ten meters each. Twenty-five plants per quadrat, making a total of 100 plants per sub-plot were randomly assessed for *B. fusca* egg batches. All egg batches collected were kept individually in small round plastic containers and brought to the laboratory at Nkolbisson. After counting the eggs, they were kept until larvae or parasitoid emergence. Parasitism was calculated as percentage of parasitized egg batches and of parasitized eggs. The parasitoids were identified using the keys in Polaszek (1998).

At 56, 84 and 112 DAP (i.e., mid-season, green and mature harvest, respectively), ten plants per quadrat were sampled at random and dissected for assessment of stem borer larvae and pupae. The number of larvae and pupae were counted according to borer species per plant. All larvae of a species from the same quadrat were placed in a container of 13 cm height and 11 cm diameter and reared in the laboratory on stem and cob pieces of maize until pupa formation or parasitoid emergence. Larvae that died during rearing were placed individually in small round plastic containers for possible parasitoid emergence. The pupae were kept individually in round transparent plastic containers until adult moth or parasitoid emergence. The emerged parasitoids were preserved in 70% alcohol and sent to the International Center of Insect Physiology and Ecology (ICIPE) in Nairobi for identification.

Soil moisture and plant nutrient analyses

At 49 DAP, straight lines at 5, 10 and 15 m perpendicular to the length of each sub-plot and starting at the grass borders were delineated. Soil samples using an auger were collected starting from the middle of the two-meter grass edge, along the three right angle lines. The first sampling point in the middle of the grass border was designated as Dist 1. The next was at two meters from Dist 1 in the first row of the maize crop and thereafter at one-meter intervals for a further five meters into the maize sub-plot (Dist 2 to Dist 7). In the control plots, samples were not collected at Dist 1 as there was no grass border. At each point, three soil samples were taken at 0–10 cm, 10–20 cm and 20–30 cm, making a total of 21 and 18 samples for each soil depth per sub-plot with or without grass border, respectively. All the samples at the same distance from the grass perimeter and along the three perpendicular lines in each sub-plot were bulked separately for each soil depth, giving a total of seven and six bulked samples per soil depth for each sub-plot with or without grass border, respectively. Each bulked sample was placed in a paper bag and weighed. They were then taken to the laboratory at Mbalmayo where they were oven-dried at 65 °C until the weight was constant. The difference in weight gave the moisture content of each sample. During subsequent collection of soil samples, each perpendicular line was staggered by one meter away from the previous delineation such that at the end of the sampling period, samples had been collected from the whole sub plot. Because soil samples could not be collected in all four blocks on the same day, one block was sampled per day. Therefore, beginning 49 DAP the four blocks were sampled for four consecutive days per week, during four consecutive weeks until 70 DAP (weeks 7 to 10).

At 56 and 112 DAP, stem, leaf and grain samples of the same plants per quadrat sampled for borer counts were collected for plant analyses. The samples from two adjacent length-wise quadrats per sub-plot were bulked and two sub-samples of stems, leaves and grain were randomly taken. The stems were chopped into 10 cm pieces. Finally, subsamples of 300 g stems, 200 g leaves and 100 g grain were oven dried at 65 °C until the weight was constant. The dried samples were then ground into a fine powder for analyses of total N, P, and K at mid-season and at mature harvest.

Plant growth, damage and yield

At green and mature harvest, plant height without tassel, percent stem tunneled and cob damage by borers, percent grain fill and cob weight were measured on the same ten plants sampled per quadrat. At mature harvest, the cobs were shelled and all the grain per quadrat bulked and weighed to calculate the shelling percent. At mature harvest, the total plants in the two middle rows in each quadrat, i.e., an area of 7.5 m^2 were harvested and the cobs weighed. Per area grain yield was calculated by multiplying the shelling percent by the total cob weight.

For the second crop, the sampling procedures and data collection were the same as in the first season.

Statistical analyses

Analysis of variance (ANOVA) in the mixed model with block as random and treatment as fixed effect (SAS 1997) was used to assess if there were any treatment differences in soil moisture, plant nutrients, pest infestations, *B. fusca* egg parasitism, plant damage, and yield variables. Variables that were collected more than once during the growing season were analyzed in repeated measures over sampling dates. For analysis of yield variables, bird and rodent damage were used as covariates.

Correlation analysis and step-wise multiple regressions were run to assess association and interactions, respectively, among the variables. In the text, correlations significant at $P \leq 0.05$ were indicated with an asterisk (i.e., r*).

The data were analyzed separately for each season. For all data, counts were log and percentages arcsine $\sqrt{\%}$ transformed before analyses. The back-transformed least square means (LSM) were presented. Significance was set at $P \leq 0.05$.

Results

Effect of grass borders and fertilizer on soil moisture and plant nutrient uptake

Percent soil moisture was higher in the grass boundaries than in the maize crop in both seasons and across sampling dates, soil depth and grass species. However, the differences were significant only during the first season. Means were 25.4%, 22.9% (df = 1, 362, F = 17.1, P < 0.0001) and 21.5%, 20.6% (df = 1, 351, F = 1.75, P = 0.19) for grass border versus maize during the first and second season, respectively. There was no significant difference in soil humidity in the grass borders between grass species; means were 24.8%, 26.1% (df = 1, 186, F = 0.81, P = 0.37) and 21.9%, 21.1% (df = 1, 184, F = 0.24, P = 0.63) for *Panicum maximum* versus *Pennisetum purpureum* during the first and second season respectively. Within the maize crop and at the different soil depth, maize+Pm tended to have the highest soil moisture but the differences were significant only during the second season at 10–20 cm depth with 20.3, 21.2 and 19.3% for maize only, maize + Pm, and maize + Pp, respectively (F = 3.02; df = 2, 544, P = 0.05). Nitrogen uptake by maize tended to be highest in plots surrounded by elephant grass but the differences were significant during the second season only; in the first season, it was 0.72, 0.74 and 0.75% (df = 2, 122, F = 0.33, P = 0.72) and during the second season it was 0.67, 0.65 and 0.69% (df = 2,

135, F = 2.86, P = 0.05) with, respectively, maize only, maize + Pm and maize + Pp.

During both seasons, plant nitrogen was significantly higher in maize sub-plots that received fertilizer than the control; it was 0.80% versus 0.68% (df = 1, 122, F = 11.46, P = 0.0001) and 0.71% versus 0.63% (df = 1, 135, F = 31.37, P < 0.0001), in the first and second season, respectively. In the second season percent plant K was significantly lower in maize that received fertilizer than in the control, with 0.64% versus 0.77% (df = 1, 135, F = 4.86, P = 0.03). The grass by fertilizer interaction was significant for plant P in the second season (df = 2, 135, F = 3.62, P = 0.03); P was significantly higher in sub-plots that did not receive fertilizer, with maize + Pm having the highest uptake (LSM = 0.24%, 0.25% and 0.22% in, respectively, maize only, maize + Pm and maize + Pp plots).

Effect of treatment on pest infestation and parasitism

Five borer species were collected, namely *Busseola fusca*, *Eldana saccharina*, the noctuid *Sesamia calamistis* Hampson 1910, the pyralid *Mussidia nigrivenella* (Ragonot 1888) and the tortricid *Cryptophlebia leucotreta* (Meyrick 1913). In both seasons, *B. fusca* egg infestation was significantly higher in sub-plots that received fertilizer (tab. 1). The main plot effects were mostly not significant for borer larvae and pupae except for *M. nigrivenella* and *C. leucotreta* during the first season, with maize + Pm having the least numbers of the former and maize + Pp the least of the latter (LSM = 0.12, 0.09, 0.17 df = 2, 1905, F = 3.95, P = 0.02 for *M. nigrivenella* and 0.04, 0.06, 0.03, df = 2, 1905, F = 3.10, P = 0.05 for *C. leucotreta* for maize only, maize + Pm, maize + Pp). During both seasons, the

fertilized sub plots had about twice the number of *B. fusca* compared to the control. Similarly, *E. saccharina* numbers were almost two times higher in fertilized sub-plots in the second season (tab. 1). The grass by fertilizer interaction was significant only for *B. fusca* during the second season (df = 2, 2853, F = 5.06, P = 0.006). Maize + Pp had significantly lower numbers of borers in sub-plots that received fertilizer; the mean values were 0.38, 0.46 and 0.33 for maize only, maize + Pm and maize + Pp, respectively. In the first season, although the grass by fertilizer interaction was not significant for *E. saccharina* (df = 2, 1905, F = 1.49, P = 0.22). *E. saccharina* abundance was significantly lower in the maize + Pp treatment in the sub plots that did not receive fertilizer; the mean values were 0.08, 0.09, 0.05 in, respectively, maize only, maize + Pm and maize + Pp.

The only parasitoids obtained from *B. fusca* eggs in the two experiments belonged to Scelionids *Telenomus* spp. (Scelionidae), with exception of one egg batch during the second season which was parasitized by *Trichogramma* spp. *Telenomus busseolae* constituted over 85% of the *Telenomus* spp. The main plot treatments had no significant effect on *B. fusca* egg parasitism except in the first season when maize + Pp had the highest percentage of egg batch parasitism (df = 2, 6, F = 4.04, P = 0.05); the values were 17.8%, 21.4%, 39.9% for, respectively, maize only, maize + Pm and maize + Pp. The grass by fertilizer interaction was significant only for percent eggs parasitized in the first season. Maize + Pp had the highest percent of egg batches and eggs parasitized in the sub plots without fertilizer (tab. 2) while during the second season egg batch parasitism was highest in the pure

Table 1. Effect of fertilizer on number of *B. fusca* egg batches per plant and eggs per plant at six weeks after maize planting, number of *B. fusca* and *E. saccharina* larvae and pupae as well as borer damage during the first and second season of 2002 at Nkometou 111 Essong.

Season	Fertiliser	Egg batch/ plant	Eggs/ plant	*B. fusca*/ plant	*E. saccharina*/ plant	% Plants infested with *B. fusca*	% stem tunnelled	% cob damage
First	Applied	0.26	1.33	2.62	0.07	89.43	17.57	17.30
	None	0.20	0.93	1.42	0.07	74.33	9.20	13.92
	df	1, 2385	1, 2383	1, 2853	1, 1905	1, 270	1, 1904	1, 1905
	F-value	12.58	11.81	241.07	0.04	33.10	87.91	8.18
	P > F	0.0004	0.0006	<0.0001	0.85	<0.0001	<0.0001	0.004
Second	Applied	0.16	0.70	0.39	0.09	35.45	4.25	0.67
	None	0.10	0.42	0.16	0.05	15.24	1.35	0.32
	df	1, 2386	1, 2385	1, 2853	1, 1905	1, 273	1, 1905	1, 1905
	F-value	20.54	20.15	170.53	14.85	58.60	141.97	12.18
	P > F	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0005

Foot note: a split plot design with maize surrounded by grass border rows as main plots and fertilizer treatment as sub plot was used. Back transformed least square means after ANOVA in the mixed model and in repeated measures of the sub-plot are given.

maize treatment without fertilizer. When compared across fertilizer level, the sub-plots without fertilizer in the maize + Pp treatment had the significantly highest percent egg and egg batches parasitized as well as sex ratio (i.e. proportion of females) in the first season. In the second season, the fertilized sub plots of this treatment had the highest egg batch and egg parasitism rate (tab. 2). In both seasons, *B. fusca* larval and pupal parasitism was less than 1%. The combined larva and pupal parasitism on maize only, maize + Pm and maize + Pp treatments were, respectively, 0.22%, 0.53%, 0.21% during the first and 0.52%, 0.48%, 1.13% during the second season. In the first season, *B. fusca* pupal parasitoids constituted 55.1%, larval parasitoids 34.5% and larval-pupal parasitoids 10.4% of the species collected. The only pupal parasitoid species was *Procerochasmias nigromaculatus* (Cameron 1906) (Hym: Ichneumonidae). The larval parasitoids were all braconids. The larval/pupal parasitoid was *Tetrastichus* sp. in addition, two *M. nigrivenella* pupae were parasitized by *Tetrastichus* spp. and a Chloropidae (Diptera) respectively. *Tetrastichus* spp. can both be a primary or hyperparasitoid. During the second season, *B. fusca* pupal parasitoids constituted 37.5% and larval 62.5%. Again *P. nigromaculatus* was the only pupal parasitoid. Three of the larval parasitoids were Diptera and two Braconidae. Of the three Diptera, one was a Chloropidae and the other a Tachinidae. Parasitism was too scanty to allow for a meaingnful statistical analysis.

Effect of treatment on plant damage and yield

For most plant damage variables, maize + Pp plots tended to be the least damaged but the differences were only significant for percent cob damage in the second season (LSM = 0.69%, 0.47%, 0.31%, respectively for maize only, maize + Pm and maize + Pp; df = 2, 6, F = 4.43, P = 0.05). In both seasons, percent plants infested with *B. fusca* larvae, stem tunneling and cob damage were significantly higher in sub-plots that received fertilizer (tab. 1).

In both seasons, cob fill, per plant cob weight and per area grain weight were significantly higher in sub plots that received fertilizer than in the control. In the fertilized plots, cob weight was 1.5 and grain weight more than 2.5 times higher in the first season and about two times higher in the second season compared to unfertilized plots (tab. 3). Similarly in both seasons, the grass by fertilizer interaction was significant for the three yield variables. During both season, there were some significant differences in cob fill and weight, and grain weight/area between main (grass) treatments but there were not clear trends (tab. 4).

Interactions among variables

Since for most of the variables the main plot effects and the grass by fertilizer interactions were not significant, the correlation and multiple regression analyses were run across treatments. Also, interactions among almost all variables showed similar trends in both seasons, thus the correlations were pooled across the two seasons.

Table 2. Grass by fertilizer interaction effect on *Telenomus* spp. parasitism of *B. fusca* egg batches, *B. fusca* eggs, parasitoid sex ratio and percent *T. busseolae* on maize at six weeks after maize planting in a split plot design with maize surrounded by grass border rows as main plots and fertiliser treatment as sub plot during the first and second season of 2002 at Nkometou 111 Essong.

Season	Treatment	% Egg batches parasitized		% Eggs parasitized		*Telenomus* spp sex ratio		% *T. busseolae*	
		Fertiliser	None	Fertiliser	None	Fertiliser	None	Fertiliser	None
First	Pure maize	19.6A	16.0aA	6.7aA	2.5aA	0.92A	0.90A	99.7bA	92.2A
	Maize + Pm	16.1A	26.6aA	3.0aA	7.5abA	0.63A	0.66A	66.5aA	97.9A
	Maize + Pp	28.8A	51.5bB	5.1aA	10.1bB	0.47A	0.85B	98.6bA	100.0A
	df	2, 584		2, 584		2, 31		2, 31	
	F-value	2.17		4.20		2.00		2.09	
	P > F	0.11		0.02		0.15		0.14	
Second	Pure maize	58.0A	65.4bA	34.6abA	29.2A	0.56A	0.77A	98.0A	90.4A
	Maize + Pm	36.2A	30.3aA	14.4aA	10.5A	0.52A	0.66A	97.8A	98.0A
	Maize + Pp	59.8B	31.3aA	40.0bB	13.0A	0.71A	0.75A	98.6A	98.3A
	df	2, 388		2, 388		2, 109		2, 109	
	F-value	1.92		1.96		0.29		0.45	
	P > F	0.15		0.14		0.75		0.64	

Back transformed least square means after ANOVA in the mixed model are presented. Within season, means within row followed by the same upper case letters and means within column followed by the same lower case letter are not significantly different following pair wise comparison.

Table 3. Sub plot effect of fertilizer on the yield variables of percent cob fill, per plant cob weight, shelling percent and per area grain weight in a split plot design with maize surrounded by grass border rows as main plots during the first and second season of 2002 at Nkometou 111 Essong.

Season	Fertilizer	%Cob fill	Cob weight	Shelling percent	Grain weight
First	Applied	59.6	118.2	32.4	325.4
	None	42.4	75.5	27.3	123.4
	df	1, 1905	1,1904	1, 32	1, 23
	F-value	59.6	153.3	4.92	86.0
	P > F	<0.0001	<0.0001	0.03	<0.0001
Second	Applied	79.9	155.8	69.9	356.5
	None	69.6	97.8	69.8	189.1
	Df	1, 1905	1, 1905	1, 30	1,32
	F-value	55.5	322.7	0.01	61.7
	P > F	<0.0001	<0.0001	0.94	<0.0001

Back transformed least square means after ANOVA in the mixed model and in repeated measures across harvest dates (84 and 112 DAP) are shown.

During the mid season, all three plant nutrients were positively correlated with each other (r = 0.48* for N:P, r = 0.69* for N:K and r = 0.59* for P:K). At both mid season and harvest, soil water was negatively related to P (r = −0.40*, −0.59*). *Busseola fusca* numbers at both mid season and at harvest increased with egg infestation (r = 0.47* and 0.47*, respectively) but decreased with egg parasitism (r = −0.48* and −0.53*, respectively). Similarly, stem tunneling and cob damage increased with *B. fusca* at mid-season (r = 0.95* and 0.96*, respectively) and at harvest (r = 0.89* and 0.92*, respectively) while egg parasitism decreased it (r = −0.46* and −0.56, respectively). *Busseola fusca* numbers at harvest were negatively related to N, P and

K at mid season (r = −30*, −45*, −83*, respectively), and positively with N (r = 0.56*) and negatively with P and K (r = −0.36 and −0.45*, respectively) at harvest. Stem tunneling and cob damage increased with N and decreased with P and K at harvest (r = 0.58*, −0.31*, −0.46* for stem tunneling) and (r = 0.33*, −0.20*, −0.59* for cob damage) respectively.

Stem tunneling and cob damage also increased with *E. saccharina* (r = 33*, 25*) and cob damage with *M. nigrivenella* (r = 0.71*) infestation. Consequently, yield decreased with *B. fusca* numbers at mid season and harvest (r = −0.19*, −0.27*, respectively) as well as with *M. nigrivenella* infestations (r = −0.35*); it increased with egg parasitism (r = 0.33*), N, P and K at mid season (r = 0.59*, 0.15*, 0.45* respectively). In multiple regressions, *B. fusca* densities at harvest were positively affected by number of egg batches per plant and plant nitrogen content at 56 DAP while egg parasitism and P at 56 DAP had a negative effect (tab. 5). In addition, soil moisture and N at 112 DAP were positively related to *B. fusca* densities in the first season. Similarly during the first season, stem tunneling was positively related with *B. fusca* and *E. saccharina* numbers and plant N, and negatively with plant K and P (tab. 5c). Similar effects were found in the second season except for *E. saccharina* and P, which were not related to tunneling (tab. 5d). Cob damage followed the same trends as stem tunneling (tabs. 5e, f).

Discussion

Percent soil moisture was higher under the grass borders than in the maize crop, confirming the hypothesis that the grassy margins could enhance water

Table 4. Grass by fertilizer interaction effect on yield variables in a split plot design with maize surrounded by grass border rows as main plots and fertilizer treatment sub plots during the first and second season of 2002 at Nkometou 111 Essong.

Season	Grass border	%Cob fill		Cob weight		Shelling percent		Grain weight/area	
		Fertilizer	None	Fertilizer	None	Fertilizer	None	Fertilizer	None
First	Pure maize	63.3bB	41.0A	122.8B	70.6aA	31.7A	30.1A	337.8abB	155.0A
	Maize+ *P m*	60.6abB	39.8A	120.0B	69.0aA	36.4B	26.1A	393.2bB	107.2A
	Maize+ *P p*	54.9aB	46.4A	111.7B	86.9bA	29.1A	25.6A	245.3aB	108.0A
	df	2, 1905		2, 1904		2, 32		2, 32	
	F - value	4.01		7.06		1.58		4.06	
	P > F	0.02		0.0009		0.22		0.03	
Second	Pure maize	79.5B	69.3abA	160.0B	96.5A	72.3A	70.7A	449.1bB	229.6A
	Maize+ *P m*	80.6B	65.2aA	160.0B	92.4A	68.2A	72.4A	361.0abB	158.5A
	Maize+ *P p*	79.7B	74.1bA	147.3B	104.5A	69.0A	66.2A	259.4aB	179.1A
	df	2, 1905		2, 1905		2, 30		2, 32	
	F - value	3.84		5.82		0.61		4.22	
	P > F	0.02		0.003		0.55		0.02	

Backed transformed least square means after ANOVA in the mixed model and in repeated measures across harvest dates (84 and 112 DAP). Within season, means within row followed by the same upper case letters and means within column followed by the same lower case letter are not significantly different.

Table 5. Effect[a] (across treatment in a split plot design with maize surrounded by grass margins as main plots and fertilizer sub plots) of soil moisture, egg infestation, egg parasitism and plant nutrients on *B. fusca* infestations at harvest during (a) the first, (b) second season; and effect[a] of soil moisture, egg infestation, egg parasitism, borer numbers at harvest and plant nutrients on stem tunnelling during (c) first and (d) second, and on cob damage during (e) first and (f) second season of 2002 at Nkometou 111 Essong.

	b	F	Mean ± SE
(a) Y number of *B. fusca* at harvest			
X$_1$ % soil moisture	5.01	39.24*	23.44 ± 0.16
X$_2$ number of *B. fusca* egg batches per plant	0.01	3.24	0.33 ± 0.01
X$_3$ *T. busseolae* parasitism	−0.29	53.06*	87.42 ± 2.84
X$_4$ % stem and leaf N at 56 DAP	9.17	21.33*	1.18 ± 0.04
X$_5$ % stem and leaf P at 56 DAP	−24.48	6.41*	0.11 ± 0.00
X$_6$ % stem and grain N at 112 DAP	17.70	123.72*	0.81 ± 0.02
Intercept = −2.77, R^2 = 0.90, N = 48			
(b) Y number of *B. fusca* at harvest			
X$_1$ no of *B. fusca* egg batches per plant	−0.01	4.11*	0.19 ± 0.01
X$_2$ *T. busseolae* parasitism	−0.08	7.82*	92.90 ± 1.09
X$_3$ % stem and leaf N at 56 DAP	15.51	263.97*	1.34 ± 0.01
X$_4$ % stem and leaf P at 56 DAP	−10.43	13.05*	0.19 ± 0.00
Intercept = −0.94, R^2 = 0.76, N = 48			
(c) Y % total stem tunnelled			
X$_1$ % soil moisture	−0.43	4.26*	23.44 ± 0.16
X$_2$ number of *B. fusca* larvae and pupae at harvest	0.31	590.62*	2.49 ± 0.12
X$_3$ number of *E. saccharina* larvae and pupae at harvest	0.31	16.56*	0.13 ± 0.01
X$_4$ % stem and leaf N at 56 DAP	0.75	11.82*	1.18 ± 0.04
X$_5$ % stem and leaf K at 56 DAP	−1.23	20.71*	1.17 ± 0.03
X$_6$ % stem and grain P at 112 DAP	−3.23	16.68*	0.18 ± 0.01
Intercept = 0.43, R^2 = 0.94, N = 48			
(d) Y % total stem tunnelled			
X$_1$ no of *B. fusca* egg batches per plant	0.002	6.39*	0.19 ± 0.01
X$_2$ no of *B. fusca* larvae and pupae at harvest	0.43	354.59*	0.35 ± 0.02
X$_3$ % stem and leaf K at 56 DAP	−1.42	43.86*	1.36 ± 0.02
X$_4$ % stem and grain N at 112 DAP	2.09	12.07*	0.78 ± 0.01
Intercept = −0.05, R^2 = 0.89, N = 48			
(e) Y % cob damaged			
X$_1$ *T. busseolae* parasitism	0.08	20.09*	87.42 ± 2.84
X$_2$ number of *B. fusca* larvae and pupae at harvest	0.20	51.85*	2.49 ± 0.12
X$_3$ number of *E. saccharina* larvae and pupae at harvest	0.55	17.59*	0.13 ± 0.01
X$_4$ stem and leaf P at 56 DAP	−7.14	24.06*	0.11 ± 0.00
X$_5$ % stem and grain K at 112 DAP	−3.50	10.73*	0.60 ± 0.02
X$_6$ % stem and grain P at 112 DAP	−14.40	68.23*	0.18 ± 0.01
Intercept = 1.09, R^2 = 0.80, N = 48			
(f) Y % cob damaged			
X$_1$ no of *B. fusca* egg batches per plant	0.003	10.73*	0.19 ± 0.01
X$_2$ *Telenomus* spp. sex ratio	−0.01	3.20	0.63 ± 0.02
X$_3$ number of *B. fusca* larvae and pupae at harvest	0.20	69.21*	0.35 ± 0.02
X$_4$ % stem and grain N at 112 DAP	−1.56	6.25*	0.78 ± 0.01
X$_5$ % stem and grain K at 112 DAP	0.85	14.53*	0.64 ± 0.02
Intercept = 0.09, R^2 = 0.56, N = 48			

[a] Stepwise multiple regressions; *F values significant at P ≤ 0.05, *b* values are partial regression coefficients.

holding capacity of the soil. Thus, maize surrounded by grasses tended to have higher soil water content but the results were not consistent. Under normal rainfall conditions, the vegetation borders are not expected to have a significant influence on soil water regime (Hauser *et al. in press*). By contrast, high rainfall would

lead to leaching and loss of soil nutrients whereas drought stress would result in lack of uptake of water and soil nutrients and consequently reduced plant vigour and yield (Liptay *et al.* 1998; Traore *et al.* 2000; Kefale *et al.* 2003). Thus, during the second cropping season of 1997, when there was a prolonged drought, Ndemah *et al.* (2002) observed less wilting and more vigorous plants in crops surrounded by *P. purpureum*. Unlike in 1997, rainfall during both seasons of 2002, were regular and sufficient, which might explain the inconsistent effects of the grassy border rows on soil water, nutrient uptake and on maize growth and yield. Similarly, and in contrast to the findings by Khan *et al.* (1997; 2001) and Ndemah *et al.* (2002), the grassy borders had no influence on stem borer infestations and parasitism in the maize crop. Also, in the 1996/97 experiments by Ndemah *et al.* (2002), the effect of the border rows on stem borer infestations and yield became significant only in the second and third crop, when the border rows were well established.

In surveys in 1995 (Ndemah *et al.* 2003) and the 1996/1997 trials (Ndemah *et al.* 2002), *B. fusca* egg parasitism by *Telenomus* spp. was much higher than in the present experiments (i.e., 19.9%, 66.9% in the first and second season of 1995, respectively, and 58 - 80% in the late season of 1997). Egg parasitism has been shown to be one of the key factors affecting population abundance of *B. fusca* in the humid forest of southern Cameroon (Ndemah *et al.* 2003). According to Schulthess *et al.* (2001), wild grass hosts stabilize the system for both the pest and its natural enemies, especially during the off season when the crop is not present. In Benin, relatively high parasitism was found on grasses in inland valleys, which allowed for a gradual increase in parasitization rates during the cropping season in up-land maize. In the forest zone of Cameroon, research is underway to assess the role of these inland valleys in the population dynamics of pests and parasitoids in adjacent maize fields in order to understand the variability of especially egg parasitism on maize.

In both seasons, the most important factors related with *B. fusca* abundance, plant damage and yield were plant nutrients (highest partial regression coefficients). As previously shown by various researchers, individual mineral nutrients not only affect plant growth but they may have both a negative or positive effect on stem borer bionomics, which in turn affects the yield of the plant they inhabit and dynamics of future populations of pests and natural enemies (Sétamou *et al.* 1993; Allsop *et al.* 1993; Sétamou *et al.* 1995; Sétamou & Schulthess 1995; Phelan *et al.* 1995; Ndemah 1999; Denké *et al.* 2000; Chabi-Olaye *et al.* 2004). During both season,

plants that received nitrogen had significantly higher egg loads. Similarly, Sétamou & Schulthess (1995) in surveys in maize fields in Benin found a strong positive correlation between soil nitrogen and eggs per plant laid by *S. calamistis*. Some studies show that female moths prefer to oviposit on host plants augmented with nitrogen (Wolfson 1980; Myers 1985). Singer *et al.* (1988) and Ng (1988) showed that oviposition preference and larval performance may be correlated such that females prefer the plant species on which their larvae have the greatest chance of surviving during their first 10 days of growth. This was corroborated in the present experiment by a positive relationship between plant N and *B. fusca* as well as *E. saccharina* larval densities. Similarly, Sétamou *et al.* (1993; 1995) showed that nitrogen application to maize increased survival, growth rates and fecundity of both *S. calamistis* and *E. saccharina* and Chabi-Olaye *et al.* (2004) found that planting of a leguminous cover or grain crop during the previous season significantly increased *B. fusca* densities on the subsequent maize crop as a result of increased leaf and stem nitrogen, as compared to the maize-maize rotation.

Plant K and P, at mid-season mainly, was negatively related to *B. fusca* and *E. saccharina* abundance and to stem and ear damage, and posititvely to yield. Similarly, Ndemah (1999) found a negative relationship between *B. fusca* numbers and soil K during surveys in farmers' fields in Cameroon. Life table studies carried out on plants subjected to varying K fertilizer dosages by Denké *et al.* (2000) yielded a curvilinear relationship between K dosages and intrinsic rate of increase and a strong negative relationship with fecundity of both *S. calamistis* and *E. saccharina*. Potassium is reported to affect herbivore survival and development by its effects on plant morphology and metabolism (Perrenoud 1990). A sufficient K and P supply tends to harden plant structures. The hardening of plant structures is generally considered to improve mechanical resistance to feeding by insects (See overview by Perrenoud 1990). Also, an adequate K nutrition increases the content of total phenols and ortho-dihydroxy phenols in plants. Phenols play a beneficial role in host plant resistance (Perrenoud 1990; Price 1997). An insufficient K supply results in the accumulation of soluble N-compounds and carbohydrates and this is often accompanied by increased herbivory (Trolldonier & Zehler 1976). For diapausing species under isolated conditions such as forest fields (Ndemah 1999), K may thus have a long-term effect on population densities via reduced fecundity.

Both *B. fusca* and *E. saccharina* numbers negatively affected grain fill and yield. The former indicates

destruction of the tassel before fertilization could occur. Egg parasitism, on the other hand, had a negative effect on *B. fusca* numbers and a postitive effect on yield corroborating results by Ndemah *et al.* (2001a & b; 2003) and emphasizing again the importance of especially *T. busseolae* as a natural control factor. Presently, considerable emphasis is given in East Africa to using wild host plants to control stem borers on crops (Khan *et al.* 1997; 2001). The present findings show that the results can be very variable. While earlier trials with border rows of grasses in Benin and Cameroon showed to considerably reduce pest densities and in the latter case increased yields, the present trials yielded no advantage over maize with grassy border rows. It is hypothesized that the amount of precipitation during the cropping season and especially around tasseling of maize, size of the plots, and duration of the experiment might affect the efficiency of the technology. Whatever the reasons, the outcome of the technology is too unpredictable to be recommended to farmers, especially in areas where animal husbandry does not co-occur with cereal production and the planting of grasses has no additional benefits.

References

Allsop P.G., Sullivan G.T., Haysom M.B.C., Morgan T.A. 1993. Relationship of edaphic factors, location and harvest date to population levels of *Saccharicoccus sacchari* (Hemiptera: Pseudococcidae) on sugarcane. *Environmental Entomology* 22: 1278-1284.

Bosque-Pérez N.A., Schulthess F. 1998. Maize: West and Central Africa. 530 pp. *in*: **Polaszek A. (ed.)**, *African cereal stem borers: economic importance, taxonomy, natural enemies and control.* CAB International, Wallingford, Oxon, United Kingdom. 592 p.

Cardwell K.F., Schulthess F., Ndemah R., Ngoko, Z. 1997. A systems approach to assess crop health and maize yield losses due to pests and diseases in Cameroon. *Agriculture Ecosystems and Environment* 65: 33-47.

Chahi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2004. Effects of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in the humid forest of southern. *Agriculture, Ecosystems and Environment* 108: 17-28.

Cbabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005a. Abundance, dispersion and parasitism of the noctuid stem borer *Busseola fusca* (Fuller) in maize in the humid forest zone of southern Cameroon. *Bulletin of Entomological Research* 95: 169-177.

Chabi-Olaye A., Nolte, C., Schulthess F., Borgemeister C. 2005b. Relationships of maize intercropped and stem borer damage to maize yield and land-use efficiency in the humid forest of Cameroon. *Bulletin of Entomological Research* 95: 417-427.

Denké D., Schulthess F., Bonato O., Smith H. 2000. Effet de la fumure en potassium sur le développement, la survie et la fécondité de *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) et de *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Insect Science and its Application* 20: 151-156.

Hauser S., Nolte C. 2002. Biomass production and N fixation of five *Mucuna pruriens* varieties and their effect on maize yields in the forest zone of Cameroon. *Journal of Plant Nutrition and Soil Science* 165: 101-109.

Hauser S., Henrot J., Hauser A. 2002. Maize yields in mulched and burned *Mucuna pruriens* var. *utilis* and *Pueraria phaseoloides* relay fallow systems in southern Cameroon. *Biological Agriculture and Horticulture* 20: 243-256.

Hauser S., Norgrove L., Duguma B., Asaah E. Soil water regime under rotational fallow and alternating hedgerows on an Ultisol in southern Cameroon. *Agro-forestry Systems* (*in press*).

Kefale D., Ranamukhaarachchi S., Zobisch M. 2003. *Impact of high temperature and drought stress induced at different growth stages on flowering phenology of three maize (Zea mays) varieties.* Technological and Institutional Innovations for Sustainable Rural development, Deutscher Tropentag, Gottingen.

Khan Z.R., Chiliswa P., Ampong-Nyarko K., Smart, L.A., Polaszek A., Wandera J., Mulaa M.A. 1997. Utilization of wild gramineous plants for management of cereal stem borers in Africa. *Insect Science and its Application* 17: 143-150.

Khan Z.R., Pickett J.A., Wadhams L., Muyekho F. 2001. Habitat management strategies for the control of cereal stem borers and striga in maize in Kenya. *Insect Science and its Application* 21: 375-380.

Liptay A., Sikkema P., Fonteno W. 1998. Transplant growth control through water deficit stress – A Review. *Horticulture Technology* 8: 340-343.

Mokwunye, A.E. & Vlek, P.L.G., 1986. Management of nitrogen and phosphorus fertilizers in sub-Saharan Africa p. 173-224 *in Developments in Plant and Soil Sciences 24.* Dordrecht, *The Netherlands, Kluwer Academic Publishers.*

Myers J.H. 1985. Effect of physiological conditions of the host plant on the ovipositional choice of the cabbage white butterfly, *Pieris rapae. Journal of Animal Ecology* 54: 193-204.

Ndemah R. 1999. *Towards developing an integrated crop management strategy for the African stalk borer, Busseola fusca (Fuller) (Lepidoptera: Noctuidae) in maize systems in Cameroon.* Ph.D thesis, Hannover University, Faculty of Horticulture, Institute of Plant Diseases and Plant Protecrion, Germany 136 p. WWW.gartenbau.uni-hannover. de/ipp/entomol/ndemah/ndemah.html.

Ndemah R., Gounou S., Schulthess F. 2002. The role of wild grasses in the management of lepidopterous stem borers on maize in the humid tropics of Western Africa. *Bulletin of Entomological Research* 92: 507-519.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Cardwell K.F. 2001a. Distribution, relative importance and effect of lepidopterous borers on maize yields in the forest zone and mid-altitude of Cameroon. *Journal of Economic Entomology* 94: 1434-1444.

Ndemah R., Schulthess F., Poehling H.-M., Borgemeister C. 2001b. Natural enemies of lepidopterous borers on maize and elephant grass in the forest zone of Cameroon with special reference to *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 91: 205-212.

Ndemah R., Schulthess F. 2002. Yield of maize in relation to natural field infestations and damage by lepidopterous borers in the forest and forest/savannah transition zones of Cameroon. *Insect Science and its Application* 22: 183-193.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Poehling M., Cardwell K.F. 2003. Factors affecting infestations of the stalk borer *Busseola fusca* (Fuller) on maize in the forest Zone of Cameroon with special reference to scelionid egg parasitoids. *Environmental Entomology* 32: 51-60.

Ng D. 1988. A novel level of interactions in plant-insect systems. *Nature* 334: 611-613.

Perrenoud S. 1990. *Potassium and plant health.* International Potash Institute Research Topics, No. 3, 365 pp.

Phelan P.L., Mason J.F., Stinner B.R. 1995. Soil fertility management and host preference by European corn borer, *Ostrinia nubilalis* (Hübner), on *Zea mays* L.: a comparison of organic and conventional chemical farming. *Agriculture, Ecosystems and Environment* 56: 1-8.

Polaszek A. 1998. *African cereal stem borers; economic importance, taxonomy, natural enemies and control.* CAB International in association with the ACP-EU Technical Centre for Agricultural and Rural Co-operation (CTA), 530 p.

Price P.W. 1997. *Insect ecology.* John Wiley and sons, Inc., 605 Third Avenue, New York, Third edition. 874 p.

SAS 1997. *SAS/STAT Software: Changes and Enhancements through Release 6.12.* SAS Institute Inc., Cary, North Carolina, 1167 p.

Schulthess F., Chabi-Olaye A., Goergen G. 2001. Seasonal fluctuation of *Sesamia calamistis* (Lepidoptera: Noctuidae) in maize fields in southern Benin. *Biocontrol Science and Technology* 11: 765-777.

Sétamou M., Schulthess F. 1995. The influence of egg parasitoids belonging to the *Telenomus busseolae* (Hym.: Scelionidae) species complex on *Sesamia calamistis* (Lepidoptera: Noctuidae) populations in maize fields in southern Benin. *Biocontrol Science and Technology* 5: 69-81.

Sétamou M., Schulthess F., Bosque-Pérez N.A., Thomas-Odjo A. 1993. Effect of nitrogen and silica on the bionomics of *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 83: 405-411.

Sétamou M., Schulthess F., Bosque-Pérez N.A., Thomas-Odjo A. 1995. The effect of stem and cob borers on maize subjected to different nitrogen treatments with special reference to *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Entomologia Experimentalis et Applicata* 77: 205-210.

Singer M.C., Ng D., Thomas C.D. 1988. Heritability of oviposition preference and its relationship to offspring performance within a single insect population. *Evolution* 42: 977-85.

Traore S.B., Carlson R.E., Pilcher C.D., Rice M.E. 2000. Bt and non-Bt maize growth and development as affected by temperature and drought stress. *Agronomy Journal* 92: 1027-1035.

Trolldonier G., Zehler E. 1976. Relationships between plant nutrition and rice diseases. Fertilizer use and plant health. *Proceedings 12ᵗʰ Coll. Internat. Potash Inst., Bern*, 85-93.

Van den Berg J., Rebe M., de Bruyn J., van Hamburg H. 2001. Developing habitat management systems for gramineous stem borers in South Africa. *Insect Science and its Application* 21: 381-384.

Wolfson J.L. 1980. Oviposition response of *Pieris rapae* to environmentally induced variation in *Brassica nigra. Entomologia Experimentalis et Applicata* 27: 223-232.

Relationships of soil fertility and stem borers damage to yield in maize-based cropping system in Cameroon

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Abstract. Field trials were designed to investigate the effect of direct nitrate fertilisation and mucuna fallow on maize yield and borer attacks in the humid forest zone of Cameroon. A traditional maize-cassava-groundnut system (farmers' practice) was compared with a maize-cassava + 120 Kg N ha^{-1}, a rotation system in which maize-cassava followed a mucuna fallow as well as with a maize monocrop grown after mucuna fallow and with a maize monocrop grown with 120 Kg N ha^{-1}. Average egg batch densities of *Busseola fusca* (Lepidoptera: Noctuidae) were lower by 35-55% in inter- than monocrops but the effect of nitrogen on *B. fusca* oviposition was not different from that of mucuna fallow. Highest larval infestations were found in sole maize with nitrogen and sole maize after mucuna. Yield losses were 4-10 times higher in the farmers' practice compared to maize-cassava after mucuna and maize-cassava with nitrogen. Mixed cropping systems including farmers' practice yielded higher total gross and net benefits compared to sole maize crops. But, they were higher in maize-cassava intercrop + N than for farmer's practice.

Résumé. Interactions entre fertilité du sol et dommages créés par les foreurs sur le rendement dans un système de culture du maïs au Cameroun. Des essais en champ ont été conçus pour étudier l'effet direct de la fertilisation azotée et de la jachère de mucuna sur le rendement de maïs et les attaques de foreurs dans la zone de forêt humide du Cameroun. Le système traditionnel d'association de maïs-manioc-arachide (pratique paysanne) a été comparé aux systèmes maïs-manioc + 120 kg de N ha^{-1}, un système de rotation dans lequel l'association maïs-manioc a suivi une jachère de mucuna, une monoculture de maïs planté après une jachère de mucuna et une monoculture de maïs planté avec 120 kg de N ha^{-1}. Les densités moyennes des plaques d'œuf de *Busseola fusca* (Lepidoptera: Noctuidae) étaient de 35-55% inférieures dans les associations de cultures que dans les monocultures, mais l'effet de la fertilisation azotée sur la ponte de *B.fusca* n'était pas différent de celui de la jachère de mucuna. Les infestations larvaires les plus élevées ont été rencontrées dans les monocultures de maïs avec la fertilisation azotée et les monocultures de maïs plantés après la jachère de mucuna. Les pertes de rendement étaient 4-10 fois plus élevées dans le système de culture des paysans par comparaison aux associations de cultures maïs-manioc après mucuna et maïs-manioc avec la fertilisation azotée. Les bénéfices totaux (brut et net) sont plus élevés avec les associations de cultures y compris la pratique paysanne par comparaison aux monocultures de maïs. Cependant, ils étaient plus élevés dans le système de culture maïs-manioc + fertilisation azotée par comparaison avec la pratique paysanne.

Keywords: Stem borer, mixed cropping, soil fertility, cover crop, Africa.

Maize is the most important cereal crop grown in Cameroon and cultivated in all agro-ecological zones. In the humid forest zone of southern Cameroon maize is the third most important food crop after plantain and cassava (Enyong 1990; Almy *et al.* 1990). Farmers around the big cities of Yaoundé and Douala are market-oriented, and since the fall in cocoa prices, maize has become an important source of cash income. The major constraints to maize production in the humid forest zone of southern Cameroon are low

soil fertility and lepidopteran stem borers, especially *Busseola fusca* (Fuller 1901) (Lepidoptera, Noctuidae), which attack the crop at every growth stage. Ndemah *et al.* (2003) identified several factors that might be responsible for the temporal and spatial fluctuations of borer densities, which were later investigated in great detail in a habitat management program developed in 2001 (Chabi-Olaye *et al.* 2005a). Major findings of his study indicated that crop-plant diversity could be a key part of a strategy in controlling stem borers on maize. In addition, the integration of grain legumes or cover crops as short fallow was shown to improve the supply of mineral N in the soil and consequently the nutritional status of the maize plant and its capacity to compensate for pest damage (Chabi-Olaye *et al.*

2005c). Improving soil fertility can thus be a very effective means of complementing integrated control of stemborers in the humid forest zone of Cameroon.

Because of complexity of the pest problems and difficulties related to technology transfer, the most profitable technologies identified in Chabi-Olaye *et al.*'s (2005a) study were tested in a farmer's field together with farmer practices in the present study.

Materials and methods

Experimental site

All the experiments were conducted at Nkometou (4° 05'N, 11° 33'E), a village 40 km west of Yaoundé. Experiments were carried out during the first and second growing seasons of 2004 (herewith referred to as experiment I and II, respectively). The first and second rainy seasons last from mid March to mid July and from mid August to end of November, respectively. Average annual precipitation is 1500 mm. The trials were planted in a 2–3 year-old bush fallow, dominated by *Chromolaena odorata* (L.) R. M. King & H. Rob. (Asteraceae), on a Rhodic Kandiudilt soil. The soil physical and chemical proprieties were similar to those reported by Chabi-Olaye *et al.* (2005c). The chemical analysis of the top soil (0-20 cm) revealed a pH (H_2O) of 5.6, 0.13% total N, 1.85% organic carbon, 11.3 µg g^{-1} available P (Mehlich-III extract), and 0.23 cmol(+) 100 g^{-1} of exchangeable K.

Experimental procedure

The most profitable cropping systems identified in previous intercropping, fertilizer and cover crop trials (Chabi-Olaye *et al.* 2005a) were compared with a traditional system where maize was intercropped with cassava and groundnut. Thus, two field trials (the first in the long and the second in the short rainy season) were set up in 2004. In experiment I, the crop mixtures consisted of i. maize-cassava-groundnut, ii. maize-cassava planted in alternate hills with and without 120 kg N ha^{-1}, in which maize was planted 10–12 days after cassava and iii. sole maize with 120 kg N ha^{-1}. For assessment of the effect of reduced borer densities on maize yield, the pure maize stand and all intercrops were grown with and without an insecticide, given a total of eight treatments. All treatments were arranged in a completely randomised block design with four replications. Plots were 6 x 12 m each. The farmer's fields were 15 x 15 m larger each, and were established by farmers themselves. The four blocks were established at 150–200 m distance from each other to reduce interactions between treatments. The distance between plots within a block was 1.5 m.

For experiment II, The cropping systems tested in experiment I were selected and two cropping systems were added *i.e.*, sole maize and a maize-cassava intercrop grown after a mucuna fallow, both grown with and without an insecticide, given a total of 12 treatments in experiment II. Like in the first experiment, the 12 treatments were arranged in a completely randomised block design with four replications. Mucuna was planted between 7–10 March and left to grow from March to August of the same year, thus covering the long rainy season. The succeeding maize crop was sown between, 13–15 September of the same year. Mucuna was planted at 25 by 50 cm with 8 grains m^{-2}. The cover crops were cut about four to five weeks before planting of the succeeding maize crop, and their biomass retained on the plots without incorporation into the soil.

In the pure stand, maize planting was done at a spacing of 75 cm between and 50 cm within rows, with a plant populations adjusted to 53333 plant ha^{-1}. Planting densities were approximately 20000 and 5000 plants ha^{-1} for maize and cassava, respectively, in the intercropping treatments. The planting pattern was similar to that described by Chabi-Olaye *et al.* (2005b). In the farmer's fields, maize, cassava and groundnut was planted at 25000, 10000 plants ha^{-1}, and 91.2 kg seeds ha^{-1}, respectively in irregular manner, where up six maize plants were left per hill.

In the insecticide plots, maize plants were treated 35 and 49 days after planting (DAP) with carbofuran at ca. 1.5 a.i. kg ha^{-1} by placing the granules in the whorl of the plant. The plots were kept weed-free.

Data collection

The percentage ground cover of mucuna was estimated at four months after planting and at harvest. Ground cover was measured using a pin-point technique. A string marked at every 10 cm was placed across the two diagonals of the plot. A metal rod with a pin-head was used to touch the string at each 10 cm mark. Points, where the pin-head touched mucuna), were counted as part of the area covered by the plant. Ground cover was thereafter calculated as: total number of times a mucuna plant was touched divided by the total number of 10 cm markings times 100.

The aboveground biomass was estimated from the centre 3 by 8 m sub-plot, and a sub-sample of 200 g per plot was weighed, dried in an oven at 70 °C for 48 hours for determination of final weight. The fixed N of five-month mucuna cover crop was calculated after Tian *et al.* (1999) and Cadisch *et al.* (1989).

During the vegetative stage, 80 and 40 maize plants/plot were checked weekly on all plots in the monocrops and intercrops, respectively, for assessment of stem borer egg batches. Batches collected were brought to the laboratory for egg counts.

Larval densities were evaluated in insecticide-free plots only, using a destructive sampling method. 24 and 12 plants were randomly sampled per plot in monocrops and intercrops, respectively. Sampling started 42 days after planting and was continued at biweekly intervals until maturity of maize ears. At each sampling date, the number of plants showing borer damage was recorded, and each maize plant was dissected and the larvae and pupae of the same borer species per plant from the same plot were counted and placed together in wide-mouth jars. Borer tunnel length (cm) and percentage of plants with dead-heart symptoms were also recorded.

At harvest, yield parameters were gathered on all plots. Each plot was divided into four quadrants, and a predetermined sub-plot of 1.5 for our treatments and 9 m^2 for farmer's practice was harvested from each. In the maize plots, cobs were removed, after counting the plants, dehusked and weighed. A sub-sample of 5 cobs per quadrant was weighed, dried in the oven and the dry grains removed and weighed to determine grain dry matter yield. The total dry matter content of plant components (leaves, stems, cobs, and dry weight of grains) was also assessed from four plants.

For groundnut, the total weight of pods per plot was recorded, and seeds were removed and weighed. A sub-sample of 100 g of seeds was weighed, and dried to assess the grain dry matter yield per plot.

The total dry matter (leaves, stem and root) produced by cassava was first recorded four months after planting (4 MAP), in both experiment I and II. At final harvest at 12 months, cassava yield was determined as both fresh and dry root weight, in experiment I only. In experiment II, cassava root weight was not harvested due to financial constrain. Plants were uprooted and the storage roots weighed. From each plot, a sub-sample of about 2 kg was taken from different roots and dried in the oven at 105 °C for estimation of root dry matter (DM). The total root dry matter was estimated by multiplying the fresh root weight with the proportion of dry matter.

Statistical analyses

The analyses were done separately for each experiment. Differences in total dry matter, Crop yields, and damage variables were analyzed by analysis of variance (ANOVA), using the general linear model (GLM) procedure of SAS (SAS 1997). *LSD* values at 5% significant level were computed. The variation in *B. fusca* abundance (eggs and larvae) over sampling days was analyzed by ANOVA, using the mixed model procedure of SAS with repeated measures (SAS 1997). The treatments were considered as fixed effects, while plants within replications were considered as random factor. Least squares means (LSM) were separated using the t-test. The significance level was set at $P = 0.05$.

Maize grain yield losses due to stem borer were assessed on an area basis as follows:

$$100 \times (Y_i-Y_t)/Y_i$$

where Y_i and Y_t are the mean yields of protected and non-protected plots, respectively.

The total gross benefit and net benefit were calculated for each treatment using the average crop yield. The following prices were taken for calculating the total gross benefit and net benefit: maize = 0.2778 $ US kg^{-1}, cassava = 0.0926 $ US kg^{-1} fresh roots; groundnut= 0.6944 $ US kg^{-1}, urea = 0.4259 $ US kg^{-1} and carbofuran = 4.6296 $ US kg^{-1}, with 1 US-$ = 540 FCFA.

Sensitivity analysis was undertaken to help identify the key variables that can influence the adoption of different cropping systems. The estimated net benefit calculated for each cropping system with the above mentioned prices represent our base scenarios that assume a specific level of technologies efficiency. The sensitivity analysis involves recalculating the net benefit for different values of crop and inputs prices, which varied one at a time. In total, there were four major alternate scenarios that could produce a significant difference in adoption of the proposed technologies: (a) maize price increase and double (100% increase); (b) groundnut price increase and double; (c) fresh root cassava price decrease by up to 70%; (d) Prices of inputs (fertilizer and insecticide) increase and double.

Results

Soil coverage and nitrogen accumulated in the mucuna biomass

The five-month mucuna cover crop provided an almost complete soil cover (96.3 to 99%) in all fallow plots (tab. 1). The residues did not disintegrate much following the short dry spell, and therefore provided good soil cover into the fall. The amount of biomass produced by the 5-month mucuna fallow did not differ

Table 1. Ground cover, the aboveground dry matter (DM-AB) and fixed N of 5-month Mucuna cover crop in field trials established in the long rainy season of 2004.

Treatments[1]	Gound cover	DM-AB	Estimated fixed
	(%)	(t ha^{-1})	N (kg ha^{-1})[2]
Sole maize-IP	97.3 ± 1.1	4.419 ± 0.204	141.4 ± 6.5
Sole maize-TP	99.0 ± 0.6	4.453 ± 0.031	142.5 ± 1.0
Maize/cassava-IP	96.3 ± 0.8	4.334 ± 0.172	138.7 ± 5.5
Maize/cassava-TP	97.0 ± 1.2	4.432 ± 0.156	141.8 ± 4.9
P-value	0.266	0.951	0.950

[1]IP and TP referred to infested and insecticide-treated plot, respectively;
[2]Fixed N: assuming N concentration in aboveground vegetation is 4% (Tian et al. 1999) and N fixation is 80% (Cadisch et al. 1989).

significant among plots (tab. 1) and ranged between 4.33 and 4.45 t ha^{-1}. For a given fallow treatment, the average subsequent N fixed was estimated at 141.1 kg ha^{-1}.

Seasonal fluctuation of stem borer infestations on maize in the different cropping systems

No difference in egg batch distribution was found between insecticide-free and treated plots
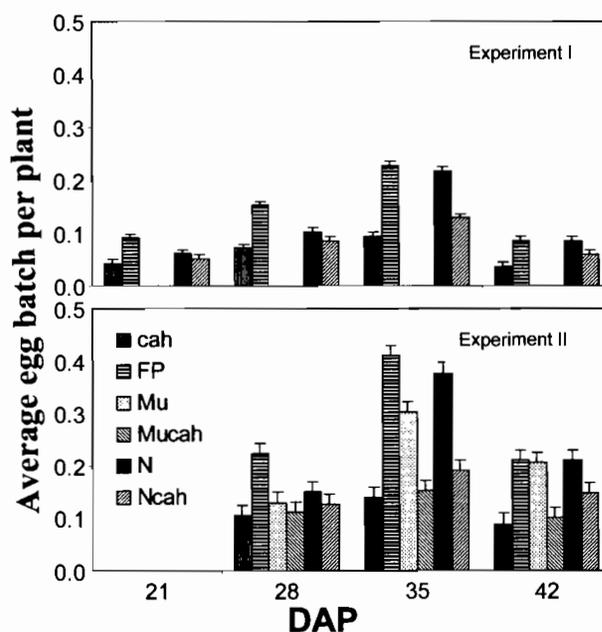
Figure 1
Seasonal fluctuation of egg batches in the different maize-based cropping systems (data pooled across insecticide-free and treated plots) (DAP, days after planting; FP, farmer's practice (maize-cassava-groundnut in an irregular pattern); N, sole maize with additional mineral fertilizer; Mu, sole maize grown after mucuna fallow; Cah, maize-cassava intercropping in alternate hill; Ncah, maize-cassava intercropping + additional mineral fertilizer; Mucah, maize-cassava intercropping grown after mucuna fallow).

Table 2. Least squares mean of egg batch per plant and % infested plant in the different maize-based cropping systems, across sampling days.

Treatments	Egg batch per plant[1]	% Infested plants (PI)[2]
Experiment I		
Sole maize+N	0.116a	21.5a
Maize/cassava	0.061b	11.3b
Maize/cassava+N	0.082b	13.7b
Farmer's practice	0.139a	19.1a
S.E	0.012	1.4
d.f.	60	60
Experiment II		
Sole maize+N	0.246ab	38.2ab
Sole maize-after Mucuna	0.213b	34.4b
Maize/cassava	0.111c	20.5c
Maize/cassava+N	0.154c	23.7c
Maize/cassava- after Mucuna	0.122c	23.2c
Farmer's practice	0.281a	41.4a
S.E.	0.022	1.5
d.f.	66	66

[1], Average over treated and non-treated plot; [2], Estimated at 49 days after planting on non-treated plots. Within column, for a given experiment, means followed by the same lowercase letter are not significantly different at $P = 0.05$ (*t*-test); d.f.: degrees of freedom in the *F*-test.

($P > 0.5$). So data were pooled across treated and non-treated plots. However, *Busseola fusca* egg densities varied significantly with days after planting in both experiments (linear trend between 21 and 42 DAP, $F = 355.85$, $P < 0.001$, for experiment I, and between 28 and 42 DAP, $F = 137.68$, $P < 0.001$, for experiment II). While no eggs were collected at 21 DAP in experiment II, the average egg batch densities was < 0.1 per plant in experiment I, increased with DAP and peaked at 35 DAP, in both experiments (fig. 1). The average percentage of plant infested per plot and egg batch densities per plant differed significantly ($P < 0.001$) among cropping patterns, and were significantly lower in maize-cassava intercrops compared to sole maize, in both experiments (tab. 2). By contrast, no significant differences in the percentage of plants infested per plot and egg batch densities per plant were found between sole maize treatments and the farmer's practice (tab. 2). The reduction in percentage of plant infested and egg batch densities were, respectively, 32.3–44.1 and 35.2–51.8%, in maize-cassava intercrops compared to sole maize treatments in experiment I, and 38.0–46.3 and 37.4–54.9% in experiment II.

Table 3. Effects of different cropping systems on populations of *Busseola fusca* and damages caused by the borer to maize.

Treatments	*Busseola fusca* per plant			Plant damage variables		
	Vegetative	At harvest		% Dead-heart[1]	Tunneling (cm)	
		IP	TP		IP	TP
Experiment I						
Sole maize+N	1.72a	0.50	0.09	3.2b	22.6	5.3
Maize/cassava	1.10c	0.37	0.10	2.7b	11.0	4.7
Maize/cassava+N	1.36b	0.46	0.09	3.5b	12.9	5.5
Farmer's practice	1.41b	0.36	0.07	9.7a	18.2	4.2
P-value	0.002	<0.001		<0.001	<0.001	
S.E.	0.083	0.021		0.576	1.122	
d.f.	12	24		12	24	
Experiment II						
Sole maize+N	3.21a	0.78	0.28	3.4b	32.9	8.4
Sole maize-after Mucuna	2.46b	0.63	0.25	2.7b	24.8	6.7
Maize/cassava	1.35d	0.52	0.25	4.1b	14.6	6.2
Maize/cassava+N	2.00c	0.71	0.21	4.1b	18.2	5.9
Maize/cassava- after Mucuna	1.81c	0.48	0.19	3.1b	16.3	6.2
Farmer's practice	2.38b	0.57	0.27	11.2a	24.2	5.2
P-value	<0.001	<0.001		<0.001	<0.001	
S.E.	0.098	0.056		0.586	1.466	
d.f.	18	36		18	36	

[1], Estimated at 49 days after planting on insecticide-free plots. Within column, for a given variable, means followed by the same lowercase letter are not significantly different at $P = 0.05$ (*t*-test); d.f.: degrees of freedom in the *F*-test; DAP: days after planting of maize.

In both experiments, number of *B. fusca* larvae per plant differed significantly among treatments during the vegetative stage at 42 DAP (tab. 3) and it was highest in sole maize with 120 kg N ha^{-1} and lowest in the maize-cassava intercrop without N. At harvest, there were no clear trends between unprotected treatments and differences between treatments in the protected plots were not significant. However, depending on cropping system they were 2–6 times higher in insecticide-free plots compared to insecticide-treated plots (tab. 3).

In experiment II, where maize was planted during the short rainy season, larval densities were 1.5–2.0 times higher compared to experiment I, where maize was planted in the long rainy season.

Effect of different cropping patterns on plant damage by B. fusca

In both experiments, a significantly higher percentage of dead heart was found in the farmer's practice treatment (tab. 3). No significant difference in percentage of dead heart was found between sole maize and the maize-cassava intercrops, and their average were, respectively, 2.7 and 4.1 times lower than in the farmer's practice. At harvest, the average plant height did not differ significantly ($P > 0.5$) among treatments. However the average was reduced by 1.5–2% in insecticide-free plots compared to treated plots in both experiments. The amount of plant tunneling differed significantly among treatments in insecticide-free but not in the insecticide-treated plots (tab. 3). In insecticide-free plots, the highest and lowest tunneling were found in sole maize and maize-cassava mixed cropping systems, respectively, in both experiments.

Crop yields and maize yield losses in the different cropping patterns

Dry matter of maize and cassava differed significantly ($P < 0.001$) among treatments (tab. 4). Maize dry matter in the insecticide-treated plots did not differ significant from that of insecticide-free plot in all treatments except for the farmer's practice, where they were 44.2% and 38.1% times higher in the insecticide-treated than insecticide-free plot, in, respectively, experiment I, and II.

In both experiments, the highest cassava dry matter at four months after planting was found in maize-cassava cropped with additional fertilizer, and averages were 38.7% higher than that of the farmer's practice in experiment I and 65.3% higher in experiment II.

There was no significant difference in groundnut yields between insecticide-treated and free plots, but the average yield was 18.2% higher in experiment II

Table 4. Effects of different cropping systems on maize and cassava total dry matter production (t ha^{-1}).

Treatments	Maize DM at harvest		Cassava DM at 4 MAP[1]	
	IP	TP	IP	TP
Experiment I				
Sole maize+N	14.055	14.348	-	-
Maize/cassava	6.633	7.588	3.080	3.130
Maize/cassava+N	8.083	8.516	4.024	4.131
Farmer's practice	2.400	4.301	2.485	2.513
P-value	<0.001		<0.001	
LSD (5%)	1.743		0.458	
d.f.	24		18	
Experiment II				
Sole maize+N	12.658	13.528	-	-
Sole maize-after Mucuna	9.583	9.976	-	-
Maize/cassava	4.040	4.290	2.251	2.277
Maize/cassava+N	5.960	6.159	3.224	3.354
Maize/cassava- after Mucuna	5.010	5.197	2.660	2.847
Farmer's practice	2.073	3.350	1.117	1.163
P-value	<0.001		<0.001	
LSD (5%)	1.251		0.300	
d.f.	36		24	

[1]Month after planting.

than in experiment I. The highest cassava root yield (root DM) at harvest was found in the maize-cassava intercrop planted with mineral fertilizer (tab. 5). The average cassava yield in the farmer's practice was twofold lower than in maize-cassava intercrop planted with mineral fertilizer. However, maize grains yields differed significantly ($P < 0.001$) among treatments in both experiments. Depending on soil fertility level, the average maize yield in the maize-cassava intercrops, with and without N, was, respectively, 49.3 and 53.0% higher compared to farmer's practice in experiment I, and 41.1 and 55.2% higher in experiment II. Hence, maize yield losses due to borer attack were 3.6 and 4.7 times higher in farmer's practice than in the sole maize planted with mineral N, in experiment I and II, respectively. In the mixed cropping systems, the lowest yield losses were found in the maize-cassava + N in experiment I and in maize-cassava planted after mucuna, in experiment II.

Economics analyses

In both insecticide-free and treated plots, maize-cassava mixed cropping systems including the farmer's practice yielded higher total gross benefits and net

Table 5. Effects of different cropping systems on dry grains and cassava root yields[1] as well as maize yield loss due to stemborers.

Treatments	Maize		Cassava		Groundnut		Maize yield loss (%)
	IP	TP	IP	TP	IP	TP	
Experiment I							
Sole maize+N	5.100	5.525	-	-	-	-	7.7
Maize/cassava	1.884	2.193	4.808	4.884	-	-	14.1
Maize/cassava+N	2.268	2.358	6.124	5.958	-	-	3.8
Farmer's practice	0.805	1.111	2.811	2.873	0.914	0.938	27.5
P-value	<0.001		<0.001		0.792		
LSD (5%)	0.308		0.589		0.216		
d.f.	24		18		6		
Experiment II							
Sole maize+N	3.981	4.239	-	-	-	-	6.1
Sole maize-after Mucuna	3.484	3.539	-	-	-	-	1.6
Maize/cassava	1.389	1.629	nh	nh	-	-	14.7
Maize/cassava+N	1.985	2.142	nh	nh	-	-	7.3
Maize/cassava- after Mucuna	1.680	1.731	nh	nh	-	-	2.9
Farmer's practice	0.684	0.959	nh	nh	1.113	1.152	28.7
P-value	<0.001				0.634		
LSD (5%)	0.272				0.192		
d.f.	36				6		

[1], maize, groundnut and cassava fresh root were harvested at 110, 95 and 352 days after planting; nh: not harvested; IP and TP referred to infested and insecticide-treated plot, respectively; % yield loss was calculated as (TP-IP/TP)*100.

Table 6. Economic analysis of the improved cropping systems compared with farmer's practice in the humid forest of Cameroon (data pooled across seasons).

Treatments	Maize yield	Cassava yield	Groundnut yield	Total gross benefit	Cost of fertilizer	Cost of furadan	Establishment of Mucuna	Net benefit
1. Insecticide-free plots								
Sole maize+N	4.5399	0	0	1261.0833	111.1111	0	0	1149.9722
Sole maize-after Mucuna	3.4835	0	0	967.6389	0	0	37.0370	930.6019
Maize/cassava	1.6368	16.0243	0	1938.3981	0	0	0	1938.3981
Maize/cassava+N	2.1263	20.4129	0	2480.7222	111.1111	0	0	2369.6111
Maize/cassava-after Mucuna	1.6798	nh	0		0	0	37.0370	
Farmer's practice	0.7448	9.3706	1.0133	1778.2176	0	0	0	1778.2176
2. Insecticide-treated plots								
Sole maize+N	4.8820	0	0	1356.1111	111.1111	69.4444	0	1175.5556
Sole maize-after Mucuna	3.5393	0	0	983.1389	0	69.4444	37.0370	876.6574
Maize/cassava	1.9111	16.2785	0	2038.1296	0	26.0417	0	2012.0880
Maize/cassava+N	2.2499	19.8584	0	2463.7130	111.1111	26.0417	0	2326.5602
Maize/cassava-after Mucuna	1.7313	nh	0		0	26.0417	37.0370	
Farmer's practice	1.0351	9.5769	1.0450	1899.9722	0	26.0417	0	1873.9306

Yields are in t ha^{-1} and costs estimated in US Dollar; nh: not harvested
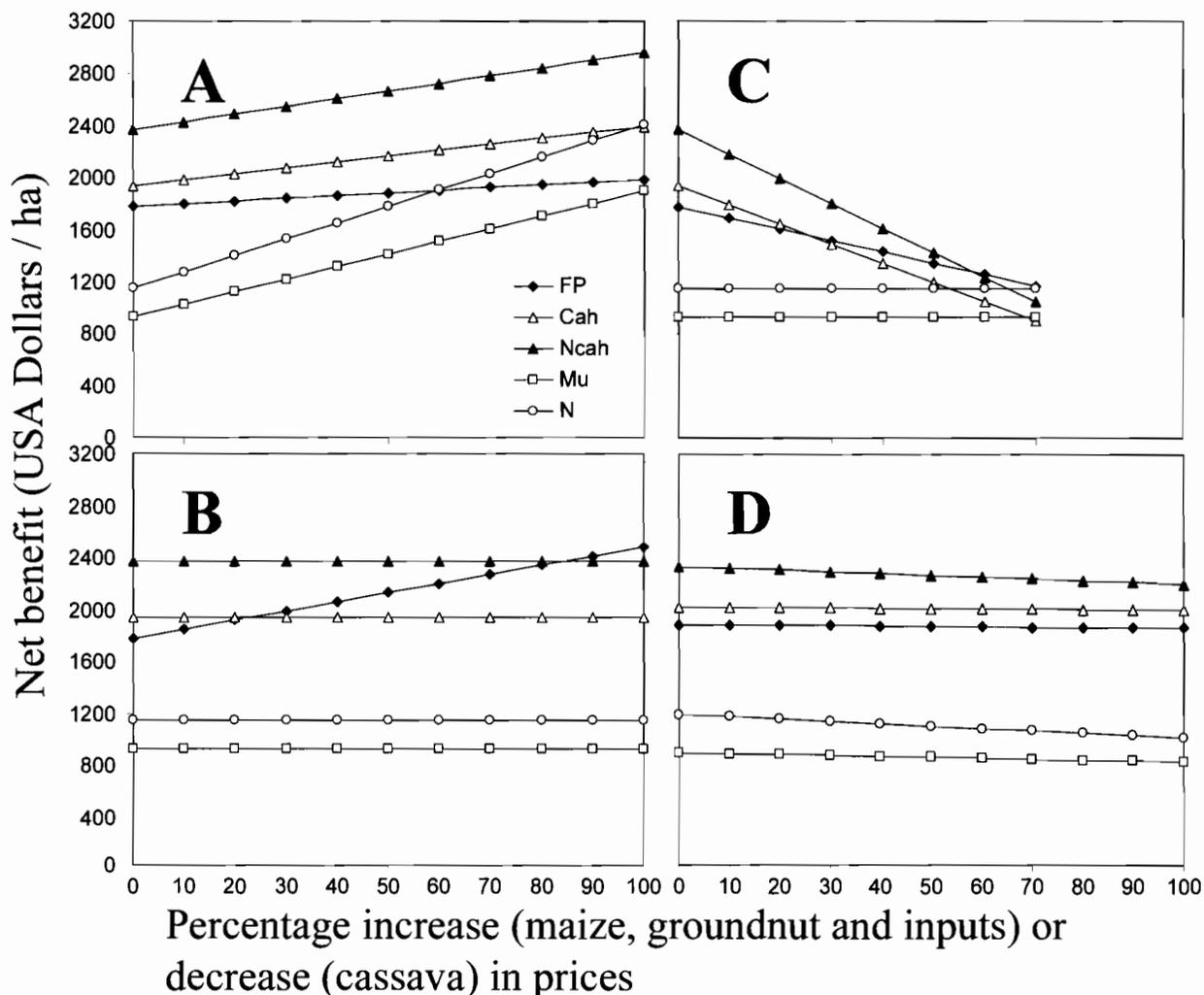
Figure 2
Sensitivity of net benefit estimates to changes in maize (A), groundnut (B), cassava fresh root (C) and inputs (D) prices (FP, farmer's practice (maize-cassava-groundnut in an irregular pattern); N, sole maize with additional mineral fertilizer; Mu, sole maize grown after mucuna fallow; Cah, maize-cassava intercropping in alternate hill; Ncah, maize-cassava intercropping + additional mineral fertilizer).

benefits compared to the sole maize cropping system (tab. 6). However, the total gross benefit and net benefit in the maize-cassava intercrop + N were 28.3 and 25.0%, respectively, higher compared to the farmer's practice, in insecticide-free plots, and 22.9 and 19.5%, respectively, higher than farmer's practice in insecticide-treated plots.

Variation in maize price (> 60% increase) produced a significant change in the net benefit of sole maize planted with additional fertilizer over that of the farmer's practice. With an 100% increase in maize price the net benefit of sole maize + N would be 17.7% higher than that of the farmers practice and lower than that of maize-cassava intercrop with N (fig. 2A). An

increase in groundnut price > 80% would produce a positive change in the net benefit of the farmer's practice over all the proposed technologies (fig. 2B). Likewise, a decrease in cassava fresh root price up to 70% would favor farmer practice over the proposed technologies (fig. 2C). Any increase in prices of inputs would slightly reduce the net benefit, but would not changes the trend found between treatments (fig. 2D).

Discussion

In the humid forest zone of Cameroon, two cropping seasons are followed by a prolonged dry season of up to four months, which causes high mortality of immature *Busseola fusca* and an extended diapause of the larvae

leads to smaller adult size, and thus, reduced female fecundity during the onset of the next rainy season (Ndemah *et al.* 2000; 2003; Chabi-Olaye *et al.* in press). Consequently, borer infestations in experiment I, set up in the first season of 2004, was lower than in experiment II, which was planted in the second rainy season. However, the present findings show that colonization of the plant by borers and severity of infestations also strongly depend on the cropping system and soil fertility which affects the nutritional status of the plant, and especially nitrogen.

In the monocrops after mucuna and with N fertilizer, survival of young larvae during the vegetative growth was positively related to soil fertility level, suggesting that higher N content in leaves lead to a higher arrestment or higher survival of young larvae. In the present experiment, larvae densities during the vegetative period were strongly related to the original egg densities. However, as shown by Chabi-Olaye *et al.* (2005c), the effect of N on *B. fusca* infestations decreased with age of the plant till tasseling, where no difference in borer numbers was found between fertilized and non-fertilized plots. Bonato *et al.* (1999) showed that nitrogen content in maize stems and leaves increased until the canopy was fully developed and then decreased with a concomitant increase of nitrogen in husks and grain. Thus the effect of nitrogen in the stems on development and survival of borers feeding in the stem decreases with age of the plant. The dynamics of nitrogen in the plant might also be the reason, why at a later stage many borers are found feeding in the ear: They follow an N gradient in the plant.

By contrast, in the mixed cropping system differences of pest infestation was mainly due to differences in eggs oviposited by the female moth with higher densities on monocropped maize. The importance of plant biodiversity in maize agro-ecosystems for reducing borer's infestation on maize has long been recognized in sub-Sahara Africa (Adesiyun 1979; Schulthess *et al.* 2004; Chabi-Olaye *et al.* 2005b & d). For the noctuids *Sesamia calamistis* Hampson 1910 and *B. fusca*, Schulthess *et al.* (2004) and Chabi-Olaye *et al.* (2005b) suggested that the presence of the non-host cassava reduced the host finding ability of the ovipositing moth, supporting the *disruptive crop hypothesis* (Root 1973; Vandermeer 1989). By contrast, in the present experiment egg densities were highest in the farmer's practice treatment where up 6 plants were left per hill. This arrangement considerably facilitated host finding by the ovipositing female moth and decreased migration related mortality of young larvae. Upon hatching, first instar larvae of *B. fusca* migrate to the whorl, where they either bore into the

stem from the top after feeding on the whorl leaves, causing the characteristic 'windows', or disperse to other plants. In contrast to non-hosts in the system, the present arrangement of many maize plants per hill increases the chance of the dispersing larvae to land on a suitable plant species. Similarly, Chabi-Olaye *et al.* (2005d) found a higher larval density on plants planted in rows than alternate hills, whereby every maize plant was surrounded by a non-host species. As results, the percentage of dead heart in farmer's practice treatment were significantly higher compared to sole maize and maize-cassava relay intercrop. This lead to a considerably higher maize yield loss in mixed cropping of farmer compared to sole maize grown on fertile soil. For *Chilo partellus* (Swinhoe 1885) (Lepidoptera: Crambidae), Mgoo *et al.* (this issue) reported that while grain weight increased with nitrogen treatments, yield losses decreased in a linear manner.

Mixed cropping systems including the farmer's practice yielded higher total gross and net benefits compared to sole maize cropping systems. This corroborates findings by Chabi-Olaye *et al.* (2005d) who showed that the net production of mixed cropping systems was considerably higher than that of pure maize stands treated with carbofuran. The sensitivity analysis showed that the estimated net benefit resulting in the adoption of one of the cropping systems tested varied widely with the price of the commodity and it was higher when both cassava and groundnut were used as companion crops.

Results of the present study clearly indicate that managing pests in the diverse maize systems in the humid forest zone of Cameroon will require multiple and integrated control practices tailored to the needs of farmers with varying production goals. Intercrops will certainly constitute a key component in any strategy to control maize stem borers in this region. Direct application of N or use of short fallow of leguminous cover crops in reducing yield losses due to borers in the subsequent crop could form a strong component of such an IPM package against *B. fusca*. Finally, the companion crop has to be a crop with high value to facilitate the adoption of mixed cropping systems.

References

Adesiyun A.A. 1979. Effects of intercrop on frit fly, *Oscinella frit*, oviposition and larval survival on oats. *Entomologia Experimentalis et Applicata* 24: 204-766.

Almy S.W., Besong M.T., Bakia B. 1990. *Food prices in Southwest province. Two years report. 1988-90.* Testing and Liaison Unit (TLU), National Cereals Reasearch and Extension (NCRE) project, Institute of agronomic Research, PMB 25 Buea, S.W.P., Cameroon.

Bonato O., Schulthess F., Baumgärtner J. 1999. Simulation model for maize crop growth based on acquisition and allocation processes for carbohydrate and nitrogen. *Ecological Modelling* **124**: 11-28.

Cadisch G., Sylvester-Bradley R., Nosberger J. 1989. 15N-based estimation of nitrogen fixation by eight tropical forage-legumes at two levels of P: K supply. *Field Crops Research* **22**: 181-194.

Chabi-Olaye A., Borgemeister C., Nolte C., Schulthess F., Gounou S., Ndemah R., Sétamou M. 2005a. *Role of habitat management technologies in the control of cereal stem and cob borers in sub-saharan Africa.* Presented at 2nd International Symposium on Biological Control of Arthropods (ISBCA), Davos, Switzerland 12-16 September 2005.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005b. Abundance, dispersion and parasitism of the noctuid stem borer *Busseola fusca* (Fuller) in maize in the humid forest zone of southern Cameroon. *Bulletin of Entomological Research* **95**: 169-177.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005c. Effects of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in the humid forest of southern. *Agriculture, Ecosystems and Environment* **108**: 17-28.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005d. Relationships of intercropped maize, stem borer damage to maize yield and land-use efficiency in the humid forest of Cameroon. *Bulletin of Entomological Research* **95**: 417-427.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2006. The role of inland valleys in the invasion of upland maize fields by stem borers and their natural enemies in the humid forest zone of Cameroon. *Environmental Entomology* **35**(2): 282-292.

Enyong L.A. 1990. Maize and groundnuts post harvest practices in the Centre province of Cameroon. NCRE Annual Report.

Mgoo, V.H., Makundi R. H., Pallangyo B., Schulthess F., Nanqing J., Omwega C.O. 2006. Yield loss due to the stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) at different nitrogen application rates to maize. *Annales de la Société Entomologique de France* **42**(3-4): 487-494.

Ndemah R., Schulthess F., Poehling H.-M., Borgemeister C. 2000. Species composition and seasonal dynamics of lepidopterous stem borers on maize and elephant, *Pennisetum purpureum* (Moench) (Poaceae), at two forest margin sites in Cameroon. *African Entomology* **8**: 265-272.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Poehling H-M., Cardwell K.F. 2003. Factors affecting infestations of the stalk borer *Busseola fusca* (Lepidoptera: Noctuidae) on maize in the forest zone of Cameroon with special reference to Scelionid egg parasitoids. *Environmental Entomology* **32**: 51-60.

Root R.B. 1973. Organisation of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs* **43**: 95-124.

SAS Institute. 1997. *SAS/STAT Software: Changes and Enhancements, Through Release 6.12.* SAS Institute, North Carolina, USA.

Schulthess F., Chabi-Olaye A., Gounou S. 2004. Multi-trophic level interactions in a cassava –maize mixed cropping system in the humid tropics of West Africa. *Bulletin of Entomological Research* **94**: 261-272.

Tian G., Kolawole G.O., Salako F.K., Kang B.T. 1999. An improved cover-crop fallow system for sustainable management of low activity clay soils of the tropics. *Soil Science* **164**: 671-682.

Vandermeer J. 1989. *The ecology of intercropping,* Cambridge University Press, 237 p.

Effect of nitrogen fertilizer level on infestations of lepidopterous stemborers and yields of maize in Zanzibar

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Abstract. The effect of nitrogen levels of 0, 60, 120, and 250 kg/ha and insecticide treatment (Furadan) on population densities and parasitism of lepidopteran stemborers, and maize yields were studied in Zanzibar during 2004/05. *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) dominated by 3-fold over *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and 42 fold over *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae). Stemborer density per plant and parasitism by *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) increased with nitrogen application level. Percentage of bored internodes per plant caused by stemborer decreased with N levels during the short rainy season. Pesticide application reduced densities of all stemborer species during the short rainy season, when infestations were high. Maize yield increased 2 to 8 times with N level, compared to the zero treatment, but the effect was less pronounced in the protected plots.

Résumé. L'effet du niveau de fertilité en azote du sol sur l'infestation des foreurs de graminées et les rendements du maïs à Zanzibar. Les effets d'une fertilisation azotée du sol de 0, 60, 120 et 250 kg/ha et d'un traitement insecticide sur la densité des populations de lépidoptères foreurs et sur leur taux de parasitisme, ainsi que sur le rendement en maïs ont été étudiés à Zanzibar de 2004 à 2005. *Chilo partellus* (Swinhoe) (Lepidoptera : Crambidae) a été de loin l'espèce prédominante, par 3 et 42 fois plus abondante que *Sesamia calamistis* Hampson (Lepidoptera : Noctuidae) et *Chilo orichalcociliellus* Strand (Lepidoptera : Crambidae) respectivement. La densité de foreurs par plante et le taux de parasitisme par *Cotesia flavipes* (Cameron) (Hymenoptera : Braconidae) ont augmenté avec la fertilisation en azote du sol. Le pourcentage d'inter-noeuds forés par plante a diminué avec le taux d'azote dans le sol pendant la petite saison des pluies. Le traitement insecticide a réduit la densité de toutes les espèces de foreurs pendant la petite saison des pluies, quand l'infestation était élevée. Le rendement a augmenté de 2 à 8 fois lorsque les sols sont riches en azote, par comparaison au terrain sans azote, cependant l'effet a été moins prononcé avec les champs protégés des foreurs.

Keywords: nitrogen fertilizer, stem borers, yield, parasitism, Zanzibar.

In Zanzibar, maize yields are generally low varying between 300 and 500 kg/ha (van Keulen 1990). Lepidopteran stemborers are the most serious constraints to maize production and in 89% of the cases the losses are reported to be moderate to high (Arendse 1990). *Chilo partellus* (Swinhoe) (Crambidae) is the most abundant accounting for 75.3% of all species, followed by *Sesamia calamistis* Hampson (Noctuidae) and *Chilo orichalcociliellus* Strand (Crambidae) (Niyibigira *et al.* 2001).

Surveys in farmer's fields in West Africa showed a positive relationship between soil nitrogen and stemborer densities (Sétamou & Schulthess 1995). Laboratory studies carried out by Sétamou *et al.* (1993) showed that

nitrogen application increased survival and growth rates as well as fecundity of *S. calamistis*. It was concluded that greater use of N fertilizer in order to increase maize yields could also increase borer populations and aggravate the pest problem especially during the second planting season. Chabi-Olaye *et al.* (2005) reported that an increased nutritional status of the plants lead to an increase in borer attacks during early stages of plant growth, but that it also improved plant vigour, resulting finally in a net benefit in the form of increased grain yield. In Zanzibar, the soils in the reef are especially poor in nitrogen (Kling *et al.* 1997).

In Zanzibar, introduction biological control became more important since the government cancelled pesticide subsidies for small scale farmers, and because parasitism levels by the indigenous parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) does not exceed 4% (Niyibigira *et al.* 2001). In 1999, the braconid larval

parasitoid *Co. flavipes* was introduced into Zanzibar by the International Centre of Insect Physiology and Ecology (ICIPE) for control of *Ch. partellus*. The parasitoid reduced pest densities in coastal Kenya by 70% (Zhou *et al.* 2001; Jiang *in. lit.*). Furthermore,

Table 1. Borer density (Mean ± SE/plant) according to species at different N application rates during the short and long rains in Zanzibar, during the 2004/2005 season.

Treatment	C. partellus	S. calamistis	C. orichalco-ciliellus	# total borer
Short rains				
N0	1.0±0.2b	0.37±0.1	0.08±0.04	1.5±0.2c
N1	1.7±0.2ab	0.58±0.1	0.13±0.05	2.4±0.3b
N2	3.0±0.4a	0.59±0.1	0.11±0.05	3.7±0.4a
N3	2.1±0.3ab	0.71±0.2	0.07±0.03	3.0±0.32b
F-value	5.84	0.88	0.47	16.58
P-value	0.0006	0.452	0.700	<.0001
Long rains				
N0	0.7±0.1b	0.1±0.03	0.01±0.01	1.0±0.2
N1	1.0.±0.1ab	0.2±0.08	0.01±0.01	1.0±0.1
N2	1.3±0.2a	0.2±0.05	0.02±0.02	1.4±0.2
N3	1.1±0.1a	0.1±0.04	0.04±0.04	1.2±0.1
F-value	3.86	1.31	1.22	0.74
P-value	0.009	0.271	0.302	0.527

Means (± S.E) in the same column followed by the same letter are not significantly different (Student-Newman-Keuls multiple comparison test, P<0.05).

Table 2. Effect of pesticide (furadan) on stemborer density during short and long rainy seasons of 2004/05 in Zanzibar (N0P= Protected with furadan with zero N; N0 neither furadan nor fertilizer; N3P= 5g N per plant with Furadan; N3=5g N/plant, without Furadan).

	Mean number of borers per plant (±S.E)			
	C. partellus	S. calamistis	C. orichalco-ciliellus	# total borers
Short rains				
N0P	0.2±0.1	0.03±0.02	0.00±0.0	0.2±0.1
N0	1.0±0.2	0.37±0.10	0.08±0.0	1.5±0.2
t-value	−5.68	−3.60	−1.73	−8.86
P-value	0.0001	0.0004	0.086	0.0001
N3P	0.2±0.05	0.1±0.0	0.0±0.0	0.2±0.1
N3	2.1±0.29	1.0±0.2	0.1±0.0	3.0±0.3
t-value	−7.10	−4.28	−2.44	−9.73
P-value	0.0001	0.0001	0.016	0.0001
Long rains				
N0P	0.5±0.1	0.0±0.0	0.00±0.0	0.5±0.1
N0	0.7±0.1	0.1±0.0	0.01±0.0	1.0±0.2
t-value	−1.75	−0.92	−1.42	−3.91
P-value	0.082	−0.359	0.157	0.0001
N3P	0.2±0.1	0.03±0.02	0.01±0.01	0.23±0.06
N3	1.1±0.1	0.09±0.04	0.04±0.04	1.18±0.14
t-value	−6.87	−1.30	−1.34	−7.02
P-value	0.0001	0.196	0.183	0.0001

studies by Jiang & Schulthess (2005) indicated that N fertilizer can also increase the performance of this parasitoid.

The present study was initiated to assess the effect of different nitrogen levels and pesticide application on stemborer infestation, particularly *Ch. partellus*, and yield of maize as well as on the performance of *Cotesia* spp.

Material and methods

Study sites

Zanzibar has a lowland tropical sub-humid climate, dominated by a bimodal pattern of rain fall, influenced by the prevailing monsoon trade winds, which blow from southeast in June-September and northeast in November–February. Rainfall throughout Zanzibar varies between 1000–2500 mm/yr (Anon 2003). A long rainy season occurs between March-June and a short one between October-December. The long rains (900–1000 mm) tend to be more reliable than the more variable short rains (400–500 mm). The trial was conducted during the long and short rainy seasons at Bambi research station, Central District of Unguja island.

Procedures

The short rain experiment lasted from mid-August, 2004 to mid-December, 2004, and the long rain experiment from 21st March to end of July 2005. The field size was 40 x 36 m. It was divided into 24 plots of 6 m x 6 m each, and with a distance between plots of 2 m. Maize (cv. STAHAMILI –Z), was planted at a spacing of 30 cm within row and 60 cm between rows. Three plants were grown per hole and thinned to two at 14 days after planting (DAP). Soil samples were taken from each plot and analysed before planting, to determine uniformity of the experimental block. The soil was classified as mollic leptosol (FAO Classification) (Anon, 2004), less red (IOR 4/6). The area was under continuous cultivation, and exhibited a rather mild plough pan at 40–50 cm. The pH values show an alkaline reaction, which is typical for calcareous coral areas of Zanzibar. P_2O_5 was low due to fixation of this element with excess calcium cations in the whole profile.

Four nitrogen treatments (N0, N1, N2 and N3) i.e., 0, 60, 120 and 250 kg/ha, which was equivalent to 0, 1.13, 2.25 and 5 g N/plant, and two pesticide treatments at the lowest (N0P) and highest nitrogen levels (N3P), were applied. The treatments were arranged in a complete randomized block with four replications of each treatment. Nitrogen fertilizer in the form of granules was applied once at 18 DAP. All plots received triple super phosphate and potassium fertilizer at the rate of 5 g/plant. Furadan was applied at 35 DAP at the rate of 1.5 a.i kg/ha by placing 5 g of the granules in the soil next to each plant.

Data collection was done at the pre-tasseling and harvest stages. Fifteen plants per plot were randomly sampled. Number of stemborer larvae and pupae per borer species, plant height, basal stem diameter, stem tunnel length, exit holes and internodes bored were recorded. Percentage of tunnel length and bored internodes was determined. At harvest time, ears were dehusked weighed and then degrained for determination of grain weight. Each stemborer larva was kept individually in a glass vial (8.5 x 2.7 cm) and reared until parasitoid or adult borer emergence.

The number of male and female parasitoids were determined and specimens sent to ICIPE for species identification.

Statistical analysis

The effect of nitrogen levels on abundance of stemborer density of each species, percentage of tunnel length and borer internodes per plant during both seasons were compared using analysis of variance (ANOVA) (PROC GLM) (SAS Institute 2004) and if significant, means were separated using Student-Newman-Keuls test. The effect of pesticide on protected and non-protected plots was compared by t-test. Percentage parasitism by each parasitoid species on different host species was compared using chi-square test. Stemborer data was log $(x+1)$ and percentages square root transformed. Untransformed data were presented in the tables.

Results

Effect of nitrogen on stemborer densities

During both seasons, *Ch. partellus* by far outnumbered *Sesamia calamistis* and *Ch. orichalcociliellus* (tab. 1). During the short rainy season, *Ch. partellus* and total numbers of borers were higher in N2 and N3 than in N0 and N1. During the long rainy season, *Ch. partellus* numbers tended to increase with nitrogen levels. There was no difference between treatments in density for the other two stemborer species. Stemborer numbers were higher during the short than long rainy season.

During the short rains, furadan applications resulted in a significant decrease in stemborer densities in both N0 and N3 treatments (tab. 2). The reduction

of stemborer was greater in N3 than N0. Pesticide also reduced total stemborer numbers during long rainy season. However, there was no effect of pesticide on densities of indigenous stemborers at the different nitrogen levels during the long rains.

Parasitism, effect of N levels on Cotesia spp. parasitism, progeny and sex ratio

Six parasitoid species were recovered, which included a hyperparasitoid, *Aphanogmus fijiensis* (Ferrière) (Hymenoptera: Ceraphronidae), pupal parasitoids, *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae), *Pediobius furvus* (Gahan) (Hymenoptera: Eulophidae), *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae), *Syzeuctus ruberrimus* Benoit (Hymenoptera: Ichneumonidae) and *Stenobracon* (=*Euvipio*) sp. However, the numbers were very low, and parasitism was negligible. The exotic and indigenous larval parasitoids, *Co. flavipes* and *Co. sesamiae*, respectively, were the major parasitoid species, and both were recovered from three stemborer species (tab. 3). Parasitism of *Co. flavipes* was higher at high nitrogen levels during short rainy seasons, and it was higher than that of *Co. sesamiae*. During the short rains, only *Co. flavipes* was recovered from *Ch. partellus*. Parasitism of *Ch. partellus* by both parasitoids did not vary significantly with N treatments during both seasons. For *S. calamistis*, significant differences in parasitism levels between N levels were observed during the long rainy season. In the protected plots,

Table 3. Effect of different nitrogen level on percentage parasitism of stemborers by *Cotesia flavipes* and *C. sesamiae*.

Parasitoid	Host	N0	N1	N2	N3	χ^2	P
				Short rains			
Co. flavipes	*Ch. partellus*	3.33	9.61	5.38	7.17	3.69	0.30
	S. calamistis	0	2.80	2.8	0	5.63	0.13
	Ch. orichalcociliellus	0	0	0	37.50	115.57	0.0001
Co. sesamiae	*Ch. partellus*	0	1.44	0.83	0.30	2.79	0.43
	S. calamistis	0	1.40	2.8	4.7	6.15	0.10
	Ch. orichalcociliellus	0	0	0	0	-	-
Co. flavipes	All borer species	2.3	7.5	3.6	6.1	9.29	0.026
Co. sesamiae	All borer species	0	1.4	0.9	1.5	4.47	0.215
				Long rains			
Co. flavipes	*Ch. partellus*	5.82	6.76	6.60	5.66	0.19	0.98
	S. calamistis	0	3.22	4.54	21.42	36.69	0.0001
	Ch. orichalcociliellus	50.0	100.0	63.36	36.36	296.68	0.0001
Co. sesamiae	*Ch. partellus*	0.90	0	0.90	1.25	2.78	0.43
	S. calamistis	10.0	0	18.18	0	43.87	0.0001
	Ch. orichalcociliellus	50.0	100.0	9.00	0	416.90	0.0001
Co. flavipes	All borer species	6.0	7.8	10.6	9.5	2.42	0.489
Co. sesamiae	All borer species	2.6	1.2	2.3	1.11	1.55	0.672

Table 4. Effect of N level on progeny and sex ratio (Number of female *Cotesia*/total progeny) of *Cotesia* spp. of each parasitized host during the short and long rainy seasons.

Treatment	Progeny			
	Cotesia flavipes		*Cotesia sesamiae*	
	Short rain	Long rain	Short rain	Long rain
N0	24.0±4.1	32.3±6.5b	-	28.3±2.7
N1	28.4±2.8	35.5±3.9b	28.8±5.3	92.0±44.0
N2	31.1±4.3	53.9±6.9a	31.5±14.8	44.8±20.0
N3	43.9±6.3	32.0±3.8a	26.8±1.8	24.0±2.0
F-value	2.62	3.59	0.08	1.36
P-value	0.06	0.02	0.93	0.32
	Sex ratio			
	Cotesia flavipes		*Cotesia sesamiae*	
	Short rain	Long rain	Short rain	Long rain
N0	0.46±0.13	0.87±0.02	-	0.79±0.05
N1	0.52±0.06	0.73±0.05	0.69±0.11	0.80±0.05
N2	0.48±0.08	0.80±0.02	0.61±0.21	0.83±0.03
N3	0.84±0.22	0.82±0.03	0.68±0.10	0.62±0.34
F-value	1.36	2.24	0.08	0.66
P-value	0.26	0.09	0.93	0.60

Means (± S.E) in a column followed by the same letter are not significantly different (Student-Newman-Keuls multiple comparison test, P<0.05).

parasitism was very low or zero.

Higher progeny of *Co. flavipes* was found at higher N levels during long rainy season. No differences were observed in sex ratio and progeny of *Co. sesamiae* between N treatments during both seasons (tab. 4).

Effect of nitrogen on plant damage variables

During the short rains, percent bored internodes and tunnel length per plant was greater in the low than high N dosages, and they were higher in short rain than the long rainy season (tab. 5). No difference was observed during the long rainy season. Furadan

Table 5. Percentage of bored internodes and tunnel length per plant at different N application rates during the short and long rains of 2004/05 in Zanzibar.

	Bored internodes (%)		Tunnel length (%)	
Treatment	Short rains	Long rains	Short rains	Long rains
N0	29.7±2.0aA	9.4±1.9aB	17.6±1.6aA	3.5±0.9aB
N1	33.6±2.1aA	8.2±1.6aB	16.8±1.8aA	3.2±0.6aB
N2	27.6±2.3aA	11.9±1.6aB	14.7±1.8aA	4.8±0.7aB
N3	19.0±1.9bA	11.7±1.8aB	9.1±1.3bA	4.4±0.8aB
F-value	10.25	1.64	8.32	1.62
P-value	0.0001	0.181	0.0001	0.186

Mean (± S.E) in a column followed by the same lower case letter(s) and means in a row followed by the same upper case letter(s) are not significantly different (Student-Newman-Keuls multiple comparison test, P<0.05).

application significantly decreased percent bored internodes and tunnel length during the long rains in both the low and high nitrogen treatments (tab. 6), but the effect was not significant during the short rains.

Effect of nitrogen on yields

During both seasons, cob and grain weight increased with nitrogen level (tab. 7). Cob yield increased between 2.7 and 7.5 times with N application during the short rains, and between 2.3 to 3.8 times compared to the zero N application during the long rainy season. Yield gain of maize grain had a similar trend. During both seasons and both the N0 and N3 treatments, cob and grain weights were not affected by the Furadan treatment (tab. 8).

Discussion

The results showed that increasing nitrogen fertilizer increased the number of borers, while plant damage decreased, resulting in higher maize yields. Sétamou *et al.* (1995) also concluded that increasing N fertilizer not only increase maize yields but also pest infestations. It is well known that the nitrogen content of plants can be a crucial factor for the development and reproduction of herbivores (Strong *et al.* 1984). Saroja *et al.* (1987) reported an increase in incidence of both *Scirpophaga incertulas* Walker (Lepidoptera: Pyralidae) infestation levels as well as in yield of rice with increased N levels. Archer *et al.* (1987) found that N increased pest infestations and stem damage while P decreased it, and with combinations of N and P, pest numbers were not different from those of the control. Our results showed that maximum yield was obtained at N levels of 60 and

Table 6. Percentage of bored internodes and tunnel length per plant at different N application rates on Furadan-treated and un-treated maize plots during short and long rain seasons of 2004/05 in Zanzibar (N0P= Protected with Furadan but zero N; N0 neither Furadan nor fertilizer; N3P= 5g N & Furadan/plant; N3=5g N/plant).

	Bored internodes %		Tunnel length %	
Treatment	Short rain	Long rain	Short rain	Long rain
N0P	31.6±2.4A	2.4±0.8B	21.3±3.2A	0.8±0.3B
N0	29.7±2.0aA	9.4±1.9aB	17.6±1.6aA	3.5±0.9aB
t-value	0.60	−3.84	0.45	−3.69
P-value	0.547	0.0002	0.654	0.0003
N3P	21.7±2.1A	3.7±0.9B	13.2±1.6A	1.7±0.7B
N3	19.0±1.9bA	11.7±1.8aB	9.1±1.3bA	4.4±0.8aB
t-value	0.93	−3.96	1.57	−3.39
P-value	0.354	0.0001	0.120	0.0009

Mean (± S.E) in a column followed by the same lower case letter(s) and means in a row followed by the same upper case letter(s) are not significantly different (Student-Newman-Keuls multiple comparison test, P<0.05).

Table 7. Cob and grain weight (kg/plot [N treatment]) of maize at different nitrogen levels in different rainy seasons in Zanzibar 2004/2005. The plant density was 5.56/m².

Treatment	Short rain		Long rain	
	Cob weight (kg)	Grain weight (kg)	Cob weight (kg)	Grain weight (kg)
N0	2.0±1.0cA	1.4±0.8bA	4.3±1.0cA	2.6±0.64cA
N1	5.4±1.1bB	3.2±1.1bB	10.1±1.2bA	7.1±1.06bA
N2	13.4±1.7aA	10.8±1.2aA	12.1±2.1bA	9.3±1.89bA
N3	15.0±0.5aA	13.1±0.1aA	16.3±1.3aA	13.6±1.15aA
F-value	30.98	25.45	13.13	12.96
P-value	0.0001	<0.0001	0.0001	0.0002

Mean (± S.E) in a column followed by the same lower case letter(s) and means in a row followed by the same upper case letter(s) are not significantly different (Student-Newman-Keuls multiple comparison test, P < 0.05).

Table 8. Effect of pesticide (Furadan) application on cob weight and grain weight (kg/treatment) during the short and long rainy seasons of 2004/2005 in Zanzibar (weight per plot of 6 x 6 m²).

Treatment	Cob weight (kg/plot)		Grain weight (kg/plot)	
	Short rains	Long rains	Short rains	Long rains
N0P	6.57±2.93	5.92±0.64	4.41±2.09	2.44±0.31
N0	1.84±0.87	4.28±0.87	1.41±0.79	2.64±0.64
t-value	1.55	1.52	1.34	0.28
P-value	0.172	0.167	0.229	0.784
N3	14.88±0.47	16.34±1.27	13.05±0.48	13.56±1.15
N3P	15.31±1.64	17.04±1.87	13.33±1.52	14.08±1.55
t-value	–0.26	–0.31	–0.18	0.27
P-value	0.806	0.761	0.8640	0.795

120 kg/ha. High N dosages drastically reduced numbers of exit holes, tunneling length and bored internodes. Sétamou *et al.* (1995) found that average cob weight losses due to borer activity decreased linearly with N application. This indicated that under the prevailing damage levels, the positive effect of N fertilization enhanced plant vigor, and surpassed the negative effect of increased borer's feeding.

The pesticide applications were more effective under high than low borer densities, at both N levels. Nevertheless, the treatments applied had little effect on maize yields because it was only applied once, which was not sufficient corroborating results by Kalule *et al.* (1998) and Ndemah & Schulthess (2002). Similarly, Egwuatu (1982) reported that furadan failed to control flea beetles, *Podagrica* spp. on okra during late stages of crop development.

This study confirmed, that *Co. flavipes* has established in Zanzibar. Nitrogen application was found to increase parasitism of *Co. flavipes* corroborating results by Jiang & Schulthess (2005) who showed that brood size of *Co. flavipes* increased with nitrogen level due to an increased quality of the larval host. Sétamou *et al.* (2005) reported that the brood size of *Co. flavipes* significantly varied with the host larval size. However, no significant differences between nitrogen treatments were found in sex ratio and progeny of *Co. flavipes* corroborating results by Jiang & Schulthess (2005).

References

Anonymus 2003. Zanzibar land husbandry improvement programme strategy and action plan. *Investment Centre Division FAO/World Bank Cooperative Programme. No: 03/007 CP-URT. January*, p. 32-50.

Anonymus 2004. CTA Annual report 2004. *Report. Technical Centre for Agricultural and Rural Cooperation ACP-EU (CTA)* p. 38-39.

Archer T.L., Bynum Jr. E.D., Onken A.B. 1987. Influence of fertilizer on southwestern corn borers, *Diatraea grandiosella*, infestation and damage to field corn. *Entomologia Experimentalis et Applicata* **43**: 271-274.

Arendse P.W. 1990. Farmers' knowledge on and attitudes towards pest problem in Zanzibar (Unguja). Agriculture University Wageningen (Internal Publication).

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005. Effect of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller) (Lepidoptera :Noctuidae) in the Humid forest of southern Cameroon. *Agriculture, Ecosystems and Environment* **108**: 17-28.

Egwuatu R.I. 1982. Filed trials with systemic and contact insecticides for the control of *Podagrica uniforma* and *P. sjostedti* (Coleoptera: Chysomelidae) on okra. *Tropical Pest Management* **28**: 115-121.

Jiang N., Schulthess F. 2005. The effect of nitrogen fertilizer application to maize and sorghum on the bionomics of *Chilo partellus* (Lepidoptera: Crambidae) and the performance of its larval parasitoid *Cotesia flavipes.* (Hymenoptera:Braconidae). *Bulletin of Entomological Research* (in press).

Kalule T., Ogenga-Latigo M.W., Okoth V.A.O. 1998. Efficacy of different insecticides for the management of stemborers of maize in Uganda. *African Crop Science Journal* **6**: 103-108.

Kling J.G., Oikeh S.O., Akintoye H.A., Heuberger H.T., Horst W.J. 1997. Potential for developing nitrogen use efficient maize for low input agricultural systems in the moist savannas of Africa. p. 490-501 in: G.O Edmendes *et al.* (ed.), *Developing drought- and low-nitrogen tolerant maize.* Proc symp. CIMMYT. El Batan, Texcoco, Mexico. 25-29 Mar. 1996. CIMMYT, Mexico, D.F.

Ndemah R., Schulthess F. 2002. Yield of maize in relation to natural field infestations and damage by lepidopterous borers in the forest and forest/savannah transition zones of Cameroon. *Insect Science and its Application* **22**: 183-193.

Niyibigira E.I., Abdullah Z.S., Lada V.Y. 2001. Distribution and Abundance, in maize and sorghum, lepidopteron stemborers and Associated indigenous parasitoids in Zanzibar. *Insect Science and its Application* **21**: 335-346.

Saroja R.F., Jagannathan R., Raju N. 1987. Effect of N nutrition and rice variety on leaf folder (LF), yellow stemborer (YSB), and grain yield. *International Rice Research Newsletter* 12: 11-12.

SAS Institute 2004. User guide: Statistics Release V8.0. SAS institute, Cary, North Carolina.

Sétamou M., Jiang N, Schulthess F. 2005. Effect of the host plant on the survivorship of parasitized *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) larvae and performance of its larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). *Biological Control* 32: 183-190.

Sétamou M., Schulthess F. 1995. The influence of egg parasitoids belonging to the *Telenomus busseolae* (Hymenoptera: Scelionidae) species complex on *Sesamia calamistis* (Lepidoptera: Noctuidae) population in Maize fields in Southern Benin. *Biological Science and Technology* 5: 69-81.

Sétamou M., Schulthess F., Bosque-Perez N.A ., Thomas-Odjo A. 1993. Effect of plant nitrogen and silica on bionomics of *Sesamia calamistis* (Lepidoptera: Noctuidae) *Bulletin of Entomological Research* 83: 405-411.

Sétamou M., Schulthess F., Bosque-Perez N.A., Thomas-Odjo A. 1995. The effect of stem borers on maize subjected to different nitrogen and silica treatments, with special reference to *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Entomologia Experimentalis et Applicata* 77: 205-210.

Strong D.R., Lawton J.H., Southwood T.R.E. 1984. *Insect on plants. Community patterns and mechanisms.* Blackwell Scientific publications, Oxford.

Van Keulen A. 1990. Effects of fertilizer and insecticide on stem borer attack in maize under farmers' condition in Zanzibar. An on-farm trial to assess biological yield and profitability of these inputs. Department of Entomology, *Wageningen University Internal Publication* 184: 62.

Zhou G., Baumgärtner J., Overholt W.A. 2001. Impact assessment of an exotic parasitoid on (Hymenoptera: Braconidae) stemborer (Lepidoptera) population dynamics in Kenya. *Ecological Applications* 11: 1554-1562.

Yield loss due to the stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) at different nitrogen application rates to maize

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Abstract. Field trials were conducted at Kibaha and Morogoro in eastern Tanzania during two seasons to evaluate the effect of nitrogen fertilization (0, 50, 75, 100 kg [N]/ha) on pest abundance, plant damage and yield loss of maize due to stemborers. In general, ear and grain weights increased linearly with nitrogen level. In the infested plot, grain weight increased 2.5 and 1.8 fold from 0 to 100 kg [N]/ha in the short and long rainy season, respectively, at Kibaha, and 1.4 and 1.6 times at Morogoro. Yield loss decreased with an increase in nitrogen application and the effect was stronger under high than low borer infestation levels. The results show the beneficial effect of nitrogen on the plant's ability to compensate for borer damage. Analysis of economic benefits of applying fertilizer and insecticide treatment indicated that using insecticides is not profitable under high-pest-low-soil fertility conditions.

Résumé. Pertes de rendement du maïs dues au foreur *Chilo partellus* (Swinhoe) (Lepidoptera : Crambidae) à différents niveaux d'application azotée. Des essais au champ ont été menés à Kibaha et Morogoro à l'Est de la Tanzanie pendant deux saisons pour évaluer l'effet de la fertilisation azotée (0, 50, 75, 100 kg [N]/ha) sur l'abondance du ravageur, les dommages et les pertes de rendement du maïs causées par les foreurs. Généralement, le poids des épis et des grains a augmenté linéairement avec le niveau en azote dans le sol. Pour les parcelles infestées par le ravageur, le poids des grains a augmenté de 2.5 et 1.8 fois pour un traitement allant de 0 à 100 kg [N]/ha pendant la petite et longue saison des pluies respectivement, à Kibaha, et de 1.4 et 1.6 fois à Morogoro. La perte de rendement a diminué avec un accroissement de la teneur azotée dans le sol et l'effet s'est accentué lors de faible niveau de densité en foreurs. Les résultats montrent l'effet bénéfique du traitement azoté sur la capacité de la plante à compenser l'attaque des foreurs. L'analyse des bénéfices économiques sur l'utilisation d'engrais et d'insecticides a montré que l'utilisation d'insecticide dans des conditions de faible fertilité du sol lors de fortes attaques de ravageur n'était pas profitable

Keywords: stemborer, *Chilo partellus*, nitrogen, yield loss, economic analysis.

Maize (*Zea mays* L. [Poaceae]) is the principal food and cash crop for millions of people in eastern Africa. In Tanzania, maize is produced on approximately 1.7 million ha, which is about 60 % of the area planted with cereals (CIMMYT 1992). Smallholder farmers account for the largest share of maize production in the country. Maize is produced mainly under rain-fed agriculture and yields are generally low, averaging around one ton per ha, compared to a world average of 4.2 t/ha (FAO 2000). Lepidopteran stemborers are generally considered the major biotic constraint to maize production in the region (Kfir *et al.* 2002).

Surveys conducted in the country between 1980 and 2000 on maize and sorghum showed that the exotic *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) were the key pest species while *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), *Eldana saccharina* (Walker) (Lepidoptera: Crambidae), and *Chilo orichalcociliellus* (Strand) (Lepidoptera: Crambidae) were of minor importance (Nyambo & Kabissa 1988; Omwega *et al.* 1997). *C. partellus*, accidentally introduced from Asia before the 1930s, was for the first time reported in Tanzania in the early 1950s (Duerden 1953).

There are numerous studies that describe the effects of nutrients, such as nitrogen and potassium, on stemborer incidence in cereals such as rice (Saroja *et al.* 1987; Thakar & Mishra 1989; Sharma & Reddy 1991; MacLean *et al.* 2003), maize (Archer

et al. 1987; Martin *et al.* 1989; Sétamou *et al.* 1993; 1995; Ndemah 1999; Denké *et al.* 2000) and in sugar cane (Atkinson & Nuss 1989). Laboratory and field studies conducted in Benin showed that increasing soil nitrogen favoured both plant growth, and the survival and fecundity of stem-borers such as *S. calamistis* and *E. saccharina* (Sétamou *et al.* 1993; 1995; Sétamou & Schulthess 1995). Thus, although pest densities were higher, yield losses decreased with the nitrogen dosage applied (Sétamou *et al.* 1995). This was corroborated by Chabi-Olaye *et al.* (2005) for *B. fusca* in the humid forest zone of Cameroon, where planting of leguminous food and cover crops in the previous season increased nitrogen for the subsequent maize crop, which led to an increase in pest densities but at the same time reduced yield losses.

Yield losses of up to 80% due to stemborers have been reported in neighboring Kenya (Ampofo 1986; Seshu Reddy & Sum 1992), where maize grows in agro-ecological conditions similar to those found in Tanzania. In Tanzania, despite the importance of cereal stemborers, yield losses caused by stemborers and the relationship with fertilizer application have not been quantified. The present study aims at determining losses to grain of maize subjected to different nitrogen (N) fertilizer dosages due to *C. partellus* at two locations in Tanzania.

Materials and Methods

Study sites

Both sites are characterized by a bimodal rainfall distribution which splits the year into a long (February-June) and a short (September-January) rainy season. In the Coastal region, the trial was planted at the Sugarcane Research Institute, Kibaha, in a sandy loam soil (6°41.708' S, 38°41.513' E, 153 m a.s.l.), and in Morogoro at the Sokoine University of Agriculture on a clay loam soil (6°51.061' S, 37°39.336' E, 549 m. a.s.l.). Mean temperature was 26.0 °C and 25.0 °C in short and long rainy seasons in Kibaha, and 26.7 °C and 26.5 °C in Morogoro, respectively. Mean maximum temperature and rainfall were similar at both sites, while in Morogoro, the mean minimum temperature was 2 °C lower than in Kibaha.

The experimental plot contained 32 subplots of 4.5 m x 6 m each, arranged in a completely randomised block design. The 110-day maize variety TMV1, which is a high yielding, drought tolerant and maize streak virus resistant, was planted. Three maize seeds were sown per hill at a spacing of 30 cm within rows and 75 cm between rows and thinned to one 14 days after planting (DAP). Four N levels (0, 50, 75, and 100 kg [N]/ha) were chosen replicated eight times, half of which were treated with the systemic Furadan 5G while half remained untreated (control). Furadan was applied at 14 and 35 DAP by putting 0.068 g a.i./plant of granules in the funnel of the plant.

At six weeks after planting (WAP), ten plants per plot were randomly selected and dissected. Data recorded were the number of stemborers according to species, plant height, stem diameter, plant damage variables (i.e. the percentage of internodes and tunnel bored, and the number of plants with dead-heart symptoms). At harvest, percent stem tunneling and of internodes bored, and the ear and grain weights were recorded for each plant sampled. Yield loss was calculated as the percentage difference between treated and untreated maize.

Plant and soil nitrogen analysis

Soil sampling was carried out prior to planting in the short rainy season. Fifteen samples were taken at 0–25 cm depth along the diagonals and transects (five samples for each) in each main plot. Soil samples were air-dried and ground to pass a 2 mm mesh. In the laboratory the soil pH was analyzed by glass electrode method (McLean 1982) using a 1:2.5 soil-water suspension. Total nitrogen was determined using the Micro-Kjedahl distillation method (Bremner & Mulvaney 1982). Extractable phosphorus was analyzed by using the method of Bray & Kurtz as described by Nelson & Sommers (1982). Organic carbon was determined according to Walkey & Black (1934) wet digestion (chromic acid titration procedure) (Olson & Sommers 1982). Other soil chemical and physical properties including cation exchangeable capacity (CEC), electrical conductivity (EC) in 1:2.5 water extract and particle size analysis were also determined.

At 42 DAP, ten plants were randomly taken from the insecticide-treated plots of each nitrogen level treatment. The mid stem portions were oven-dried at 65 °C for three days. The dried samples were ground to pass a mesh of 0.5 mm and the total nitrogen concentration was determined according to Kjeldahl using block digestion and steam distillation (AOAC 1990). The data from the short rains in Morogoro were excluded because the samples were mishandled.

Cost-benefit analysis

The price of maize at farm gate at harvest, fertilizer and pesticide per kg in both sites was collected from the district agricultural extension offices. The price of nitrogen fertilizer of UREA (46% N) in Morogoro and Kibaha was 956.5 Tsh (Tanzania Shilling) and 1087 Tsh/kg, respectively. Furadan 5G was 5,000Tsh and 6,800 Tsh/kg in Morogoro and Kibaha, respectively. In Morogoro, the price of maize was 194.4 Tsh/kg during short and 138.9 Tsh/kg during long rainy season while in Kibaha, they were 233.3 and 205.6 Tsh/kg, respectively. The net income was estimated on a per hectare basis and converted into USD.

Data analysis

Analysis of variance (ANOVA) using the Proc GLM (SAS 1997) was used to compare plant nitrogen content, plant growth variables, damage variables, pest counts and grain weights at different nitrogen fertilizer rates. The analyses were done separately for each cropping season and location. Step-wise multiple regressions (Proc REG, SAS 1997) were used to measure the effect of nitrogen, growth variables (plant height and stem diameter), and stem damage (percentage of stem tunnel) on grain yield. Insect counts were log $(x+1)$ transformed and the percentage data was arcsine square root transformed before analysis but in the tables and figures, untransformed mean values are presented.

Table 1. Nitrogen (%N) and dry matter content (%DM) of maize stem.

	Kibaha				*Morogoro*	
	Long rain		Short rain		Long rain	
N level	%N	%DM	%N	%DM	%N	%DM
0	0.69±0.02b	8.00±0.67	0.82±0.10c	7.59±0.18	0.78±0.02c	10.60±0.23a
50	1.10±0.11a	8.50±0.36	1.04±0.04b	8.00±0.24	1.20±0.06b	10.62±0.29a
75	1.31±0.16a	7.51±0.31	1.27±0.04a	7.78±0.42	1.17±0.05b	9.97±0.33a
100	1.50±0.12a	7.27±0.68	1.34±0.09a	6.95±0.27	1.37±0.07a	8.31±0.50b
F	9.45	0.75	11.33	2.41	22.98	8.81
P	0.002	0.55	0.001	0.12	0.0001	0.003

Means (±SE) within columns followed by the same lower case letter do not significantly at $P \leq 0.05$ (Student Newman-Keul's test).

Results

Soil and plant nutrient content analysis

In Kibaha, the soil pH value was 5.62, and % N = 0.0112, while in Morogoro, it was 6.03 and 0.0291, respectively. Phosphorus and percentage of organic carbon were 8.66 mg [P]/kg, 0.743% in Kibaha, and 9.18 mg [P]/kg, 1.596% respectively in Morogoro. CEC and EC were 16.6 cmol (+)/kg, 194.7 μs/cm in Kibaha, and 20.7 cmol(+)/kg, 104.2 μs/cm in Morogoro.

Nitrogen content of the maize stem tended to increase with the nitrogen dosage (tab. 1). No differences in dry matter content were found between treatments except for Morogoro during the long rains where the highest N dosage yielded the lowest dry matter content.

Abundance of stem borers

C. partellus and *S. calamistis* were found at both sites, with *C. partellus* accounting for 95–98%. A few *C. orichalcociliellus* were recovered at Kibaha station during the long rainy season (tab. 2).

Borer densities did not vary with nitrogen treatment for any of the locations or seasons (tab. 2). *C. partellus* numbers varied between 2.2–3.2 and 0.8–1.3 per plant during the short and long rainy season, respectively, at Kibaha, and 0.03–0.1 and 0.15–0.83, respectively, at Morogoro. They were higher during the short than the long rainy season at Kibaha ($t = 6.51$, $P = 0.0001$), it was the opposite at Morogoro ($t = -4.12$, $P = 0.0001$), and higher at Kibaha than Morogoro ($t = 13.97$, $P = 0.0001$ for short rainy season, $t = 3.52$, $P = 0.0001$ for long rainy season).

Table 2. Stem borer densities/plant (means ± SE) at different nitrogen levels at pre-tasseling during two cropping seasons at two locations in Tazania.

Kibaha	Short rains		Long rains		
Nitrogen level (kg N/ha)	*C. partellus*	*S. calamistis*	*C. partellus*	*S. calamistis*	*C. orichalco-ciliollus*
0	2.15 ± 0.43	0.10± 0.08	0.75 ± 0.28	0.00 ± 0.00	0.00 ± 0.00
50	2.08 ± 0.40	0.03 ± 0.03	1.10 ± 0.35	0.00 ± 0.00	0.05 ± 0.10
75	3.15 ± 0.50	0.05 ± 0.03	0.83 ± 0.20	0.00 ± 0.00	0.00 ± 0.00
100	3.23 ± 0.74	0 ± 0	1.33 ± 0.40	0.00 ± 0.00	0.08 ± 0.06
F	1.54	0.85	0.66		1.09
P	0.21	0.47	0.58		0.36
Morogoro					
0	0.05 ± 0.35	0.00 ± 0.00	0.30 ± 0.14	0.00 ± 0.00	0.00 ± 0.00
50	0.08 ± 0.08	0.00 ± 0.00	0.15 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
75	0.03 ± 0.03	0.00 ± 0.00	0.83 ± 0.34	0.00 ± 0.00	0.00 ± 0.00
100	0.10 ± 0.08	0.00 ± 0.00	0.50 ± 0.19	0.00 ± 0.00	0.00 ± 0.00
F	0.23		2.00		
P	0.88		0.12		

Means (±SE) within columns followed by the same lower case letter are not significantly different at P≤ 0.05 (Student Newman-Keul's test).

Table 3. Plant height and stem diameter (cm) of Furadan-treated (T) and untreated (UT) maize subjected to different nitrogen application rates during two seasons and at two locations.

N-levels	Short rainy season				Long rainy season			
	Plant height		Stem diameter		Plant height		Stem diameter	
Kibaha	T	UT	T	UT	T	UT	T	UT
0	202.7 ± 4.6bA	168.2 ± 5.9bB	1.88 ± 0.04bA	1.74 ± 0.06bA	202.5 ± 2.9bA	183.6 ± 3.6bB	1.78 ± 0.03cA	1.66 ± 0.04dB
50	205.6 ± 3.1bA	167.2 ± 5.4bB	1.99 ± 0.06bA	1.87 ± 0.06bA	201.5 ± 2.8bA	191.4 ± 3.1bB	1.83 ± 0.03cA	1.77 ± 0.03cA
75	217.0 ± 3.2aA	194.1 ± 5.7aB	2.22 ± 0.04aA	2.12 ± 0.05aA	226.4 ± 2.9aA	201.1 ± 3.8aB	2.00 ± 0.03bA	1.95 ± 0.03bA
100	225.7 ± 3.8aA	201.4 ± 3.9aB	2.29 ± 0.05aA	2.25 ± 0.06aA	231.8 ± 3.4aA	210.4 ± 2.9aB	2.12 ± 0.02aA	2.05 ± 0.03aA
Morogoro								
0	237.2 ± 3.5bA	237.8 ± 2.8A	1.87 ± 0.04dA	1.95 ± 0.04bA	240.7 ± 4.0bA	235.8 ± 3.4bA	1.75 ± 0.04cA	1.75 ± 0.03dA
50	239.3 ± 3.2bA	243.5 ± 3.4A	2.02 ± 0.04cA	2.04 ± 0.04bA	238.8 ± 3.9bA	225.0 ± 4.3bB	1.87 ± 0.03bA	1.85 ± 0.03cA
75	248.9 ± 2.3aA	244.8 ± 3.5A	2.18 ± 0.04bA	2.15 ± 0.04aA	239.2 ± 4.5bA	236.1 ± 4.3bA	1.94 ± 0.04abA	1.95 ± 0.03bA
100	250.9 ± 2.9aA	246.6 ± 3.4A	2.33 ± 0. 03aA	2.22 ± 0.04aB	259.1 ± 3.5aA	252.9 ± 2.9aA	2.05 ± 0.03aA	2.04 ± 0.03aA

Means (±SE) within columns followed by the same lower case letter and within row followed by the same upper case letter are not significantly different at P ≤ 0.05 (Student Newman-Keul's test).

Nitrogen effects on plant growth and damage variables

In both protected and infested plots, plant height tended to increase with nitrogen level in both seasons and sites, except the infested plots during the short rainy season in Morogoro (tab. 3). In Kibaha only, protected plants were significantly higher than infested ones across all the seasons and nitrogen levels (tab. 3), while at both sites, stem diameter did not vary with pesticide and nitrogen treatments (tab. 3).

Percentage of internodes and tunnel bored did not differ between nitrogen levels in both seasons and

Table 4. Percentage of bored internodes a) and percent tunnel length b) (means ± SE) at different nitrogen levels of Furadan-treated (T) and untreated (UT) maize at pre-tasselling and at harvest during two cropping seasons and at two locations.

N level	Short rainy season				Long rain season			
	Pre-tasselling		Harvest		Pre-tasselling		Harvest	
a) *Kibaha*	T	UT	T	UT	T	UT	T	UT
0	0.6 ± 0.6aB	30.5 ± 3.9aA	6.5 ± 1.4aB	34.6 ± 4.4aA	0 ± 0aB	11.5 ± 3.4aA	2.3 ± 0.9aB	14.9 ± 2.3aA
50	0 ± 0aB	28.8 ± 3.9aA	6.8 ± 1.4aB	37.3 ± 4.3aA	0 ± 0aB	11.5 ± 3.1aA	2.6 ± 1.1aB	15.7 ± 2.9aA
75	0 ± 0aB	31.6 ± 3.2aA	3.6 ± 0.7aB	42.1 ± 3.1aA	0 ± 0aB	9.6 ± 2.6aA	3.8 ± 1.3aB	19.3 ± 2.7aA
100	0 ± 0aB	26.0 ± 2.6aA	5.5 ± 1.2aB	46.5 ± 3.1aA	0 ± 0aB	13.1 ± 2.6aA	1.4 ± 0.6aB	20.0 ± 2.7aA
Morogoro								
0	0 ± 0aA	1.1 ± 0.8aA	0.4 ± 0.4aA	1.7 ± 0.9aA	0 ± 0aB	1.4 ± 0.6aA	0.5 ± 0.5aB	2.3 ± 0.8aA
50	0 ± 0aA	0.3 ± 0.3aA	0.5 ± 0.5aA	1.3 ± 0.7aA	0 ± 0aB	1.1 ± 0.6aA	0 ± 0aB	2.8 ± 1.0aA
75	0 ± 0aA	0.3 ± 0.3aA	0.8 ± 0.7aA	2.7 ± 1.5aA	0 ± 0aB	2.2 ± 1.3aA	0 ± 0aB	2.5 ± 1.0aA
100	0 ± 0aA	1.2 ± 1.0aA	0.2 ± 0.2aB	4.2 ± 1.9aA	0 ± 0aB	3.8 ± 1.3aA	0.2 ± 0.2aB	3.9 ± 1.1aA
b) *Kibaha*								
0	0.2 ± 0.2aB	8.5 ± 1.3aA	2.3 ± 0.6aB	11.0 ± 1.6bA	0 ± 0aB	2.2 ± 0.9aA	0.6 ± 0.2aB	5.5 ± 1.0aA
50	0 ± 0aB	8.2 ± 1.2aA	2.5 ± 0.6aB	15.5 ± 1.9abA	0 ± 0aB	1.9 ± 0.5aA	0.8 ± 0.4aB	6.5 ± 1.4aA
75	0 ± 0aB	9.7 ± 1.4aA	1.2 ± 0.3aB	19.8 ± 1.9aA	0 ± 0aB	2.0 ± 0.6aA	1.2 ± 0.4aB	7.8 ± 1.2aA
100	0 ± 0aB	9.0 ± 1.2aA	1.8 ± 0.5aB	18.3 ± 1.8aA	0 ± 0aB	2.5 ± 0.6aA	1.8 ± 0.5aB	8.0 ± 1.3aA
Morogoro								
0	0 ± 0aA	0.1 ± 0.1aA	0.1 ± 0.1aA	0.4 ± 0.2aA	0 ± 0aA	0.1 ± 0.1aA	0.1 ± 0.1aB	0.9 ± 0.3aA
50	0 ± 0aA	0.1 ± 0.1aA	0.4 ± 0.4aA	0.3 ± 0.2aA	0 ± 0aA	0.1 ± 0.1aA	0 ± 0aB	0.9 ± 0.3aA
75	0 ± 0aA	0.1 ± 0.1aA	0.1 ± 0.1aA	0.6 ± 0.3aA	0 ± 0aA	0.6 ± 0.5aA	0 ± 0aB	1.7 ± 0.6aA
100	0 ± 0aA	0.4 ± 0.3aA	0.1 ± 0.1aB	1.3 ± 0.5aA	0 ± 0aB	0.6 ± 0.2aA	0.1 ± 0.1aB	1.8 ± 0.5aA

Means (±SE) within columns followed by the same lower case letter and within row followed by the same upper case letter are not significantly different at P ≤ 0.05 (Student Newman-Keul's test).

Table 5. Maize cob and grain yield (g/plant) at different nitrogen levels of Furadan-treated (T) and untreated (UT) maize at pre-tasselling and at harvest during two cropping seasons and at two locations.

N-levels	Short rainy season				Long rainy season			
	Cob weight		Grain yield		Cob weight		Grain yield	
Kibaha	T	UT	T	UT	T	UT	T	UT
0	163.6 ± 8.4cA	81.9 ± 9.8dB	93.2 ± 5.3bA	44.0 ± 6.2cB	129.5±7.7dA	95.3 ± 6.0dB	93.0±5.9dA	67.8±4.6dB
50	178.9 ± 6.6cA	114.5 ± 9.8cB	104.3 ± 5.0bA	62.8 ± 6.3bB	155.5±6.6cA	122.6 ± 7.2cB	108.5 ± 4.3cA	84.8±5.2cB
75	218.2 ± 6.1bA	162.6 ± 9.9bB	125.1 ± 3.7aA	95.3 ± 6.8aB	185.8±6.4bA	154.2 ± 7.9bB	130.1 ± 4.0bA	109.0±6.4bB
100	244.0 ± 9.0aA	190.4 ± 9.7aB	137.9 ± 4.9aA	111.1 ± 5.4aB	212.9±5.4aA	178.3 ± 7.6aB	146.3 ± 4.3aA	124.6±5.6aB
Morogoro								
0	167.8 ± 10.8cA	160.1 ± 9.4cA	110.1 ± 7.4cA	108.2 ± 6.9cA	174.2 ± 7.0cA	149.1 ± 7.9dB	111.0 ± 4.7bA	96.3 ± 5.7dA
50	192.4 ± 9.7bA	215.5 ± 7.5bA	123.1 ± 7.2bcA	124.7 ± 6.5bcA	187.4 ± 7.1cA	174.6 ± 8.1cA	119.9 ± 4.8bA	112.8 ± 5.3cA
75	215.5 ± 7.5bA	209.7 ± 9.1abA	138.4 ± 5.3abA	136.4 ± 5.8abA	211.7 ± 8.4bA	203.3 ± 7.1bA	138.8 ± 5.7aA	135.0 ± 4.9bA
100	240.6 ± 5.0aA	232.0 ± 6.2aA	153.0 ± 4.1aA	152.5 ± 5.3aA	235.1 ± 6.6aA	234.9 ± 7.4aA	151.0 ± 4.6aA	150.4 ± 5.2aA

Means (±SE) within columns followed by the same lower case letter and within row followed by the same upper case letter are not significantly different at P ≤ 0.05 (Student Newman-Keul's test).

locations, except for Kibaha at harvest time during the short rainy season, where percent tunneling tended to increase with nitrogen dosage (tab. 4). Stemborer damage was higher in Kibaha than Morogoro, in both seasons. In Kibaha, the damage during the short rainy season at both pre-tasseling and harvest time was higher than in the long rainy season. In both locations and seasons percent internodes and tunnel bored were significantly lower in insecticide-treated than untreated maize.

The number of „dead hearts" varied with location and season but not treatment. More dead hearts were observed during the short than the long rainy seasons at Kibaha. At Morogoro no „dead hearts" were observed during either season. Overall, the values were very low and the data are not shown here.

Nitrogen effects on maize ear and grain yields

At Kibaha, ear weight increased linearly 1.5 fold from 0 to 100 kg [N]/ha during the short season, and 1.7 fold during the long rainy season, while at Morogoro, it increased 1.4 and 1.3 times, respectively (tab. 5). Grain weight followed the same trend. Compared to untreated plots, maize ear weights in Furadan treated plots were 2.5 and 1.8 higher in short and long rainy season, respectively, at Kibaha, and 1.4 and 1.6 times, respectively, at Morogoro (tab. 5). In the short rainy season at Kibaha, the average ear weight loss due to stemborers decreased linearly with nitrogen dosage from 50 % to 22 % and grain weight loss from 53% to 19% as nitrogen dose increased from 0 to 100 kg [N]/ha, whereas during the long rainy season, they decreased from 26 % to 16 % and 27 % to 15 %, respectively (fig. 1). At Morogoro yield losses were negligible during the short rain season but during the long rainy season,

ear and grain weight losses declined linearly from 14 % to 0 % as the nitrogen dosage increased from 0 to 100 kg [N]ha (fig. 1).

Relationship between stem nitrogen content with plant growth and damage parameters

Multiple regression of grain yield (y) (g/plant) on percent stem tunneling (x_1), stem diameter (x_2) and nitrogen level (x_3) resulted in:

$Y = 28.3 - 1.9\ x_1 + 3\ 6.0\ x_2 + 0.4\ x_3$ ($R^2 = 0.35$, $P < 0.0001$ for all variables). Maize grain yield increased with stem diameter and nitrogen application rate. By contrast, percent stem tunneling reduced yield.
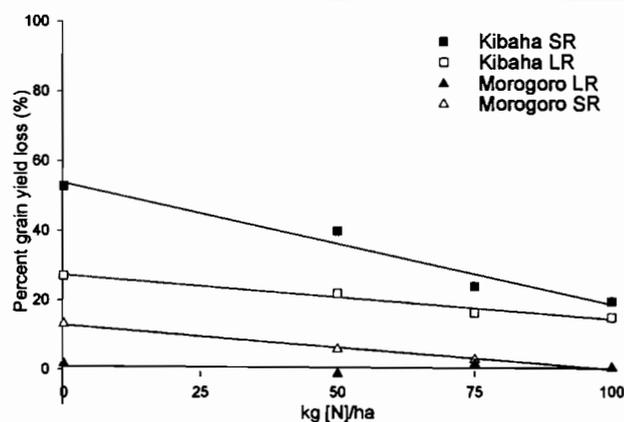
Figure 1

Percentage of grain yield loss (Y) with different nitrogen dose applications (X). SR and LR represent short and long rainy season. The solid lines represent the linear regressions of each season at each site (for Kibaha SR: $Y = 33.7 - 0.35X$, $P = 0.02$, $R^2 = 0.96$; for Kibaha LR: $Y = 27.3 - 0.13X$, $P = 0.02$, $R^2 = 0.97$; for Morogoro SR: $Y = 1.04-0.01X$, $P=0.73$, $R^2 = 0.073$; for Morogoro LR: $Y = 12.91-0.13X$, $P = 0.004$, $R^2 = 0.99$).

(Lepidoptera: Pyralidae) decreased from 20 to 11% with an increase in N fertilizer dosage from 0 to 120 kg [N]/ha. Chabi-Olaye *et al.* (2005) showed that yield losses to *B. fusca* in maize planted in continuous cropping were around 20% versus 6% and 9% in maize planted after a leguminous cover and grain crop, respectively. Similarly, in a continuous maize cropping system, yield losses due to *B. fusca* in fertilized maize was only 2–6% versus 17–25% in unfertilized plots (Borgemeister *et al.* 2005). Ndemah & Schulthess (2002) reported that the assessment of yield losses due to *B. fusca* based on total ear rather than grain weight alone, grossly underestimated the real losses. By contrast, in the present study both ear and grain losses due to *C. partellus* were very similar. According to Muturi *et al.* (2006) *B. fusca* is on an average one to two times larger than *C. partellus* and, in addition, *B. fusca* is a voracious grain feeder. While tunnel damage has a systemic effect, which affects growth of the entire ear and not only grain filling, ear feeding causes damage to the grain alone. Thus, whether total ear weight or grain alone should be used for yield loss assessments depends on the feeding habits of the borer species concerned.

The multiple regression showed that stem diameter and nitrogen application were positively, and percent tunneling was negatively, related to grain yield, corroborating results by Sétamou *et al.* (1995), Sétamou & Schulthess (1995), Gounou *et al.* (1994) and Ndemah *et al.* (2001). In general, tunnel length has shown to be a more precise and reliable measure of yield loss than insect numbers, because by the time the plants are sampled many borers might have already reached adulthood and left the plant or were killed by predators or parasitoids. Production equations, which included plant growth (growth stage and stem diameter) and borer damage (stem tunneling, ear damage) variables have been used to estimate yield losses on a regional basis via country-wide surveys (Gounou *et al.* 1994; Cardwell *et al.* 1997). As a result, growth stage x basal stem diameter has been shown to be a good indicator of plant vigor and soil fertility. This has simplified and reduced the costs of yield loss assessments.

The present findings confirm reports by Sétamou *et al.* (1995) and Chabi-Olaye *et al.* (2005) that an increased nutritional status of the plants enhanced both borer fitness and plant vigour, but with a net-benefit for the plants. Thus maintaining or improving soil fertility is an important component of integrated pest management of *C. partellus*. Furthermore, the benefits from insecticide treatments decreased with increasing fertilizer dosage, expect for Kibaha during the short

rains, when borer densities were severe suggesting that using insecticides is not profitable under high-pest-low-soil fertility conditions. Presently, a large-scale biological control (BC) program against *C. partellus* using the exotic braconid larval parasitoid *Cotesia flavipes* (Cameron) is underway in eleven countries in East and Southern Africa (Omwega *et al.* 1997; Zhou *et al.* 2001). As shown by Jiang & Schulthess (2006), nitrogen applications to the host plant *C. flavipes* is feeding upon increases the egg load of the fecundity of the progeny of the parasitoid indicating that increasing the nutritional status of the plant will also increase parasitism during the season. It is suggested that the combination of BC and technologies that improve soil fertility suffice to alleviate pest problems in maize in eastern Africa.

References

Ampofo J.K.O. 1986. Maize stalk borer (Lepidoptera: Pyralidae) damage and plant resistance. *Environmental Entomology* **15**: 1124-1129.

AOAC 1990. *Official methods of analysis.* 15th edition. Association of Official Analytical Chemist, Virginia, United States of America, 69-88.

Archer T.L., Bynum E.D. Jr., Onken, A.B. 1987. Influence of fertilizer on southwestern corn borer, *Diatraea grandiosella*, infestation and damage to field corn. *Entomologia Experimentalis et Applicata* **43**: 271-274.

Atkinson P.R., Nuss K.J. 1989. Associations between host-plant nitrogen and infestations of the sugarcane borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Bulletin of Entomological Research* **79**: 489-506.

Bonato O., Schulthess F., Baumgaertner J.U. 1999. A simulation model for carbon and nitrogen allocation and acquisition in maize. *Ecological Modelling* **124**: 11-28.

Borgemeister C., Chabi-Olaye A., Nolte C., Schulthess F., Ndemah R., Sétamou M. 2005. Role of habitat management technologies in the management of cereal stem and cob borers in sub-Saharan Africa. *International symposium on biological control of arthropods*, September 12 to 16, 2005, Davos, Switzerland, http://www.cabi-bioscience.ch./ISBCA-DAVOS-2005/scientific_program.htm.

Bremner J.M., Mulvaney C.S. 1982. Total nitrogen, p. 149–157 *in:* Page A. L. (ed.), *Methods of Soil Analysis, Part 2 Agronomy monogragh*, Number 9, American Society of Agronomy, Madison, Wisconsin.

Cardwell K.F., Schulthess F., Ndemah R., Ngoko Z. 1997. A system approach to assess crop health and maize yield losses due to pests and diseases in Cameroon. *Agriculture, Ecosystems and Enviroment* **65**: 33-47.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005. Effects of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in the humid forest of southern Cameroon. *Agriculture, Ecosystems and Enviroment* **108**: 17-28.

CIMMYT 1992. *CIMMYT World Maize Facts and Trends: Maize Research Investments and Impacts in Developing Countries.* CIMMYT, Mexico.

Denké D., Schulthess F., Bonato O., Gounou S., Smith H. 2000. Effet de la fumure en potassium sur le dévelopment, la survie et la fécondite de *Sesamia calamistis* Hampson et de *Eldana saccharina* Walker sur maïs. *Insect Science and its Application* **20**: 151-156.

Duerden J.C. 1953. Stemborers of cereal crops at Kongwa, Tanganyika 1950-1952. *East African Agricultural and Forest Journal* **19**: 105 –19.

FAO 2000. *FAOSTAT Agricultural data*, http://apps.fao.org.

Fischer K.S., Palmer A.F.E. 1984. Tropical Maize, p. 213-248 *in:* Goldsworthy P., Fisher N. M. (eds.), *The physiology of tropical field crops*, New York, John Wiley & Sons.

Gounou S., Schulthess F., Shanower T., Hammond W.H.O., Braima H., Cudjoe A.R., Adklaoe R., Antwi K.K., Olaleye I. 1994. *Stem and ear borers of maize in Ghana*. Plant Health Management Research Monograph 4, International Institute of Tropical Agriculture, Ibadan, Nigeria, 23 p.

Jiang N., Schulthess F. 2006. Effect of nitrogen fertilizer level of maize on development of *Chilo partellus* and performance of its larval parasitoid *Cotesia flavipes*. *Bulletin of Entomological Research* 16: 49-60.

Kfir R, Overholt W.A., Khan Z., Polaszek A. 2002. Biology and management of economically important lepidopteran cereal stemborers in Africa. *Annual Review of Entomology* 47:701-732.

MacLean R.H., Litsinger J.A., Moody K., Watson A.K., Libetario E.M. 2003. Impact of *Gliricidia sepium* and *Cassia spectabilis* hedgerows on weeds and insect pests of upland rice. *Agriculture, Ecosystems and Enviroment* 94:275-288.

Martin R.C., Arnason J.T., Lambert J.D.H., Isabelle P., Voldeng H.D., Smith D.L. 1989. Reduction of European corn borer (Lepidoptera: Pyralidae) damage by intercropping corn with soybean. *Journal of Economic Entomology* 82:1455-1459.

McLean F.O. 1982. Soil pH and lime requirements, p.199–224 *in:* Page A. L. (ed.), *Methods of Soil Analysis*. Part 2 Agronomy monogragh, Number 9, American Society of Agronomy, Madison, Wisconsin.

Muturi J.J., Ngi-Song A.J., Mueke J.M, Sétamou M., Schulthess F., Jiang N. Multiparasitism by the pupal parasitoids, *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae) and *Pediobius furvus* (Hymenoptera: Eulophidae) on two African cereal stemborers, *Chilo partellus* (Lepidoptera: Crambidae) and *Busseola fusca* (Lepidoptera: Noctuidae). *Biocontrol Science and Technology* (in press).

Ndemah R. 1999. *Towards developing an integrated crop management strategy* for the *African stalk borer, Busseola fusca (Fuller) (Lepidoptera: Noctuidae) in maize systems in Cameroon*. Ph.D thesis, Hannover University, Faculty of Horticulture, Institute of Plant Diseases and Plant Protection, Germany, 136 p. WWW.gartenbau.uni-hannover.de/ipp/entomol/ndemah/ndemah.html.

Ndemah R., Schulthess F. 2002. Yield of maize in relation to natural field infestations and damage by lepidopterous borers in the forest and forest/savannah transition zones of Cameroon. *Insect Science and its Application* 22: 183-193.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Cardwell K.F. 2001. Distribution, relative importance and effect of lepidopterous borers on maize yields in the Forest Zone and Mid-altitude of Cameroon. *Journal of Economic Entomology* 94: 1434-1444.

Nelson D.W., Sommers L.E. 1982. Total carbon, organic carbon and organic matter, p. 539–579 *in:* Page A. L. (ed.), *Methods of Soil Analysis*. Part 2 Agronomy monogragh, Number 9, American Society of Agronomy, Madison, Wisconsin.

Nyambo B.T., Kabissa J.C. 1988. The status of maize stalkborers in Tanzania. A review with emphasis on possible future research proposals, p. 224-228 *in:* Moshi A. J., Ramson J. K. (eds.), *Proceedings of the First Tanzania National Maize Research Workshop*, Agricultural research Organization, 6-7 June 1988, Arusha Tanzania.

Olsen S.R., Sommers L.E. 1982. Phosphorus, p. 539–575 *In:* Page A. L. (ed.), *Methods of Soil Analysis*, Part 2 Agronomy monogragh, Number 9, American Society of Agronomy. Madison, Wisconsin.

Omwega C.O., Overholt W.A., Mbapila J.C., Kimani-Njogu S.W. 1997. Establishment of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), an exotic endoparasitoid *of Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in Tanzania. *African Entomology* 5: 71-75.

Saroja R., Jagannathan R., Raju N. 1987. Effect of nitrogen nutrition and rice variety on leaffolder (LF), yellow stemborer (YSB) and grain yield. *International Rice Research Newsletter* 12: 11-12.

SAS Institute, 1997. User's guide: Statistics. SAS Institute Inc., Cary, NC, USA.

Seshu Reddy K.V., Sum K.O.S. 1992. Yield–infestation relationship and determination of economic injury level of the stem-borer, *Chilo partellus* (Swinhoe) in three varieties of maize, *Zea mays* L. *Maydica* 37: 371 –376.

Sétamou M., Schulthess F., Bosque-Perez N.A., Odjo-Thomas A. 1993. Effect of plant nitrogen and silica on the bionomics *of Sesamia calamistis* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 83: 405-411.

Sétamou M., Schulthess F. 1995. The influence of egg parasitoids belonging to the *Telenomus busseolae* (Hym.: Scelionidae) species complex on *Sesamia calamistis* (Lepidoptera: Noctuidae) populations in maize fields in southern Benin. *Biocontrol Science and Technology* 5: 69-81.

Sétamou M., Schulthess F., Bosque-Perez N.A., Thomas-Odjo A. 1995. The effect of stem and earborers on maize subjected to different nitrogen treatments. *Entomologia Experimentalis et Applicata* 77: 205-210.

Sharma A.R., Reddy M.D. 1991. Performance of different height rice lines under intermediate deep water levels. *International Rice Research Newsletter* 16: 8-9.

Simpson J.M., Simpson C.L. 1990. The mechanisms of nutritional compensation by phytophagous insects, p. 111-160 *in:* Bernays E.A. (ed.), *Insect-Plant interactions*. CRC Press, Boca Raton, Florida.

Slanksy F.J. 1993. Nutritional ecology: the fundamental quest for nutrients. p. 478-508 *in:* Stamp N. E., Casey T. M. (eds.), *Caterpillars, Ecological and Evolutionary Constraints on Foraging*. Chapman & Hall, New York.

Thakar R.B., Mishra S.S. 1989. Effect of different levels of nitrogen and some granular insecticides on stemborer and leaf folder incidence in rice. *Journal of Entomological Research* 13: 121-124.

Walkey A., Black I.A. 1934. Methods of Soil analysis. *Soil Science* 37: 29.

Zhou G., Baumgartner J., Overholt W.A. 2001. Impact of an exotic parasitoid on stem borer (Lepidoptera) populations dynamics in Kenya. *Ecological Application* 11: 1554-1562.

The role of maize-legumes-cassava intercropping in the management of maize ear borers with special reference to *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae)

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Abstract. The effects of intercropping maize with cowpea, lima bean, soybean, three leguminous cover crops (*Tephrosia vogelii* Hook F., *Canavalia ensiformis* L., *Sesbania rostrata* Bremek. & Oberm.) and cassava on the infestation of *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) and other lepidopteran ear borers were studied. Field experiments were conducted in four locations in Benin using a 4x2 pattern of maize-legumes or maize-cassava planting. Intercropping reduced the number of eggs (by >25%) and larvae (by 17.9-53%) of *M. nigrivenella* compared with the monocrop. Maize-*C. ensiformis* and maize-*T. vogelii* proved to be the most effective combinations for reducing *M. nigrivenella* populations in the different locations. Grain loss and ear damage, which were significantly correlated with the number of insects in the ear, were significantly affected by the intercrops, with losses abated by 47-84% in the four sites. No parasitized larvae were found in any of the locations.

Résumé. Le rôle des cultures mixtes maïs-légumineuse-manioc dans la gestion des mineuses d'épis de maïs en particulier de *Mussidia nigrivenella* Ragonot (Lepidoptera : Pyralidae). Les effets des cultures mixtes du maïs avec le niébé, le haricot de Lima, le soja, trois légumineuses de couverture (*Tephrosia vogelii* Hook F., *Canavalia ensiformis* L., *Sesbania rostrata* Bremek & Oberm.) et le manioc sur l'infestation de *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) et d'autres lépidoptères à chenilles mineuses de maïs ont été étudiés. Des essais au champ ont été conduits dans quatre localités du Bénin caractérisées par leurs pratiques culturales différentes, cultures mixtes de maïs avec des légumineuses ou avec le manioc. Les cultures mixtes ont permis la réduction du nombre d'œufs et de larves de *M. nigrivenella* comparativement aux monocultures. Les combinaisons de maïs cultivé avec *C. ensiformis* ou *T. vogelii* se sont avérées être les plus efficaces pour réduire les populations de *M. nigrivenella* dans les quatre localités. Les pertes de rendement et les dégâts sur épis ont été significativement réduits par la méthode de culture mixte. Aucune larve parasitée n'a été rencontrée dans les localités étudiées.

Keywords: Maize, intercropping, Canavalia ensiformis, Tephrosia vogelii, Mussidia nigrivenella.

Mussidia nigrivenella Ragonot 1888 (Lepidoptera: Pyralidae) is one of the key pests attacking maize ears in West Africa (Bosque-Perez & Mareck 1990; Moyal & Tran 1991 a, b; Shanower *et al.* 1991). It is a commonly occurring pest which causes serious damage to maize grain in the field and stores (Moyal 1988; Moyal & Tran 1991a, b; Silvie 1993). Management practices have relied on early harvesting (Sétamou M., pers. com.) and drying of the ears previous to harvesting. Trials on the use of chemicals such as deltamethrin did not produce any significant effect on the ear borer (Moyal 1988). Research on the natural enemies of *M. nigrivenella* in West Africa indicates that they are rare and not efficient (Sétamou *et al.* 2002).

In Benin, maize is traditionally planted with other crops, some of which are non-hosts of *M. nigrivenella*; this practice may reduce pest incidence on the crops

(Dissemond & Hindorf 1990; Ayisi *et al.* 2001). The only available information on the use of intercropping maize to reduce infestation by *M. nigrivenella* is the study done with peanuts by Moyal (1993 a, b), which showed no measurable effect, although cases of success have been reported for maize stem borers (Omolo 1986; Oloo & Ogeda 1990; Skovgard & Paets 1996; Paets *et al.* 1997; Schulthess *et al.* 2004).

The contribution of cover crops to the sustainability of agriculture is becoming increasingly evident in many regions of the world. Because of the great interest of West African farmers in cover crops such as *Canavalia ensiformis* L., *Tephrosia vogelii* Hook. F. and *Sesbania rostrata* Brem. & Oberm., it is expected that they will become key components of farming systems. The selection of a cover crop, however, should be based not only on its efficiency in restoring soil fertility, but also on its reactions vis-à-vis pests and natural enemies. The present study was undertaken to establish whether intercropping maize with grain legumes, cover crops and cassava can reduce the infestation by

M. nigrivenella and its damage in the maize cropping systems in different ecological zones of Benin.

Material and Methods

The trials were set up during the long rainy season of 2004 in collaboration with farmers in four locations representing three different ecological zones: (i) the International Institute of Tropical Agriculture (IITA) located in Abomey-Calavi (6°24' N, 2°24'E) in Coastal Savanna with 210 days of rainfall distributed over two cropping seasons; (ii) Cana (7°13'N, 2°07'E) and (iii) Djidja (7°33'N, 1°93'E), both in the Southern Guinea Savanna with 181 days of rainfall and two cropping seasons; and (iv) Bantè (8°42'N, 1°83'E), in the Northern Guinea Savanna with less than 150 days of rainfall and one cropping season. The following treatments were considered in each location: sole maize, maize-cowpea (*Vigna unguiculata* L. var. KVx erect variety), maize-lima bean (*Phaseolus lunatus* L.), maize-soybean (*Glycine max* L.), maize-cassava (*Manihot esculenta* Krantz) - a common combination in Benin, maize-jackbean (*Canavalia ensiformis* L.), maize-fish bean (*Tephrosia vogelii* Hook. F.) and maize-*Sesbania rostrata* Brem. & Oberm. (Leguminosae). All the leguminous plants used have been recorded as host plants of *Mussidia nigrivenella* (Sétamou *et al.* 2000a). *Canavalia*, *Tephrosia* and *Sesbania* are also used as cover crops in Benin (Carsky *et al.* 2003). The planting pattern in the intercrops was 4 rows of maize and 2 rows of legumes or cassava with spacings of 0.4 m within rows and 0.75 m between rows. Maize, legumes and cassava were sown simultaneously in a complete randomised block design with plot size of 10 x 12.75 m and 1m between plots and 2 m between blocks. The eight treatments were repeated three times. No insecticide was applied throughout the study period. Fertilizer (NPK 15-15-15) was applied two weeks after sowing and urea 45 days after sowing. The maize variety QPM (Quality Protein Maize, 110–120 days) was used.

Data collection

From the soft dough stage (approximately 70 days after sowing) to harvest, three destructive samples of 10 plants per plot were randomly taken at 2-week intervals. Ears were thoroughly examined, dissected and the numbers of *Mussidia* eggs and different stages of larvae and pupae, as well as ear damage, were assessed. Other insects found in the maize ears such as *Eldana saccharina* Walker 1865 (Lepidoptera: Pyralidae), *Sesamia calamistis* Hampson 1910 (Lepidoptera: Noctuidae) and *Thaumatotibia (Cryptophlebia) leucotreta* Meyrick 1913 (Lepidoptera: Tortricidae) were also recorded. The damages caused by the ear borers were calculated as the percentage of grains consumed and contaminated by fungi. At harvest, the percentage grain loss was estimated by the following formula: grain loss (%) = 100(Pi – Pf)/Pi, where Pi is the initial weight of the cob and Pf is the weight of the cob after the damaged grains were removed. The loss (in g) per cob is the difference between Pi and Pf. Damage by the ear borers predisposes the ears to pre- and post-harvest infestations by storage beetles, infections by fungi such as *Aspergillus flavus* and *Fusarium verticillioides* and subsequent contamination with mycotoxins. Both quality and quantity of the grains are therefore seriously affected and the damaged cobs cannot be sold nor used as food. Thus, these damaged grains were removed and considered as actual ear weight loss.

The *M. nigrivenella* larvae or pupae collected were maintained on *Canavalia* pods for recording larval or pupal parasitism. Ten ears were also selected randomly from each plot and weighed to determine the effects of each treatment on the ear weight. Additionally, 50 pods of cowpea and *Canavalia* were also randomly harvested for *M. nigrivenella* eggs and larvae; the other legumes were not yet at the fruiting stage.

Statistical analysis

Analysis of variance in the mixed model in repeated measures over sampling dates (SAS Institute 1997) was used to compare counts of immature pest stages according to borer species, plant damage and grain losses. Variables were compared between cropping systems with location, cropping system and their interactions as fixed effects. The random effects were sampling date, block (or replication) and plant. Plants were nested within treatments, treatments within blocks, blocks within location and location

Table 1. Effect of cropping systems on number of *Mussidia nigrivenella* per ear and other maize ear borer infestations, ear weight and ear damages in Bantè, Benin

Intercrop	No. of *M. nigrivenella* eggs	No. of *M. nigrivenella* larvae+pupae	No. of *Eldana saccharina*	No. of *Sesamia calamistis*	No. of other insects*	Overall ear borers	Ear weight (g)	% Ear damage	Ear loss (g)	% loss
Maize mono	0.08±0.06b	1.09±0.20b	0.29±0.14c	0.08±0.03b	0.49±0.12b	1.54±0.23b	92.68±7.76a	17.30±3.07b	10.21±2.02b	12.07±1.77b
Maize-Cass.	0.01±0.01a	0.56±0.11a	0.02±0.02a	0.06±0.02ab	0.31±0.07a	0.95±0.15a	114.84±6.46a	7.04±2.20a	6.71±1.04ab	6.38±1.57a
Maize-Cowp.	0.01±0.01a	0.61±0.14a	0.04±0.03a	0.07±0.03b	0.40±0.09b	1.12±0.16a	103.62±8.43a	9.60±2.10a	7.52±1.85ab	7.73±1.68a
Maize-Phaseo.	0.03±0.03a	0.75±0.15a	0.08±0.05b	0.08±0.03b	0.48±0.11b	1.17±0.18a	102.55±9.59a	10.38±2.04a	8.23±1.54ab	9.06±1.78ab
Maize-Soyb.	0.01±0.01a	0.57±0.12a	0.02±0.02a	0.04±0.02a	0.37±0.10b	1.00±0.21a	109.75±9.56a	9.32±2.04a	7.13±1.89ab	7.72±1.62a
Maize-Canav.	0.01±0.01a	0.52±0.09a	0.01±0.01a	0.03±0.02a	0.30±0.06a	0.86±0.16a	112.99±6.97a	7.26±1.80a	6.73±1.39ab	5.86±0.85a
Maize-Sesb.	0.06±0.03b	0.68±0.13a	0.08±0.03b	0.07±0.03b	0.47±0.09b	1.30±0.20b	98.75±7.64a	11.37±2.49ab	10.00±1.14b	9.40±1.90ab
Maize-Tephr.	0.01±0.01a	0.51±0.10a	0.00±0.00a	0.02±0.02a	0.27±0.06a	0.80±0.12a	118.86±7.00a	6.73±0.97a	4.60±1.21a	5.39±1.71a
DF**	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 230)	(7; 232)	(7; 230)	(7; 230)
P	0.020	0.048	0.006	0.004	0.028	0.049	0.290	0.006	0.016	0.024

Means within columns followed by the same letter are not significantly different (SNK, P=0.05). *Other insects are *Thaumatotibia leucotreta*, beetles (*Sitophilus zeamais* Motschulsky 1855, *Carpophilus* sp and *Cathartus quadricollis* Guérin 1829). Cass: cassava; Cowp: cowpea; Phaseo: *Phaseolus lunatus*; Soyb: soybean; Canav: *Canavalia ensiformis*; Sesb: *Sesbania rostrata*; Teph: *Tephrosia vogelii*. DF** indicates degree of freedom for both treatments and the experimental error.

Table 2. Effect of cropping systems on *Mussidia nigrivenella* and other maize ear borer infestations, ear weight and ear damages in Cana, Benin

Intercrop	No. of M. nigrivenella eggs	No. of M. nigrivenella larvae+pupae	No. of Eldana saccharina	No. of Sesamia calamistis	No. of other insects*	Overall ear borers	Ear weight (g)	% Ear damage	Ear loss (g)	% loss
Maize mono	0.21±0.21b	0.28±0.07b	0.24±0.06b	0.32±0.07 b	1.60±0.61 b	2.45±0.53b	33.38±2.53a	26.71±6.51b	4.28±1.10b	13.98±4.80b
Maize-Cass.	0.00±0.00a	0.21±0.05b	0.12±0.04a	0.23±0.07ab	0.92±0.27ab	1.47±0.29a	40.93±6.21a	17.57±4.78ab	3.10±0.68ab	8.69±1.65ab
Maize-Cowp.	0.00±0.00a	0.22±0.06b	0.19±0.05b	0.28±0.07 b	1.36±0.43 b	2.01±0.48b	35.26±3.45a	19.24±5.23ab	3.55±1.56a	10.18±3.79a
Maize-Phaseo.	0.01±0.01a	0.23±0.05b	0.22±0.04b	0.32±0.18 b	1.55±0.58 b	2.17±0.65b	31.66±2.47a	20.54±5.70ab	3.72±1.71ab	11.07±3.69ab
Maize-Soyb.	0.00±0.00a	0.15±0.04a	0.11±0.04a	0.18±0.04 a	0.90±0.23ab	1.33±0.28a	37.26±4.05a	16.72±3.11a	2.37±0.64a	8.63±2.01a
Maize-Canav.	0.00±0.00a	0.19±0.04ab	0.09±0.04a	0.17±0.04 a	0.68±0.19 a	1.13±0.16a	37.64±5.00a	15.48±4.63a	2.19±0.52a	8.34±2.88a
Maize-Sesb.	0.00±0.00a	0.21±0.05b	0.12±0.04a	0.23±0.05ab	1.54±0.47 b	2.06±0.62b	39.48±2.45a	21.60±5.48ab	3.72±1.31ab	13.85±3.35ab
Maize-Tephr.	0.00±0.00a	0.17±0.04a	0.08±0.03a	0.16±0.04 a	0.32±0.07 a	0.73±0.12a	43.86±7.66a	13.30±4.12a	1.43±0.47a	2.72±2.03a
DF**	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 222)	(7; 229)	(7; 222)	(7; 220)
P	0.0440	0.0042	0.0450	0.0050	0.0048	0.0362	0.059	0.018	0.034	0.024

Means within columns followed by the same letter are not significantly different (SNK, P=0.05). * Other insects are *Thaumatotibia leucotreta*, beetles (*Sitophilus zeamais, Carpophilus* sp. and *Cathartus quadricollis*). Cass: cassava; Cowp: cowpea; Phaseo: *Phaseolus lunatus*; Soyb: soybean; Canav: *Canavalia ensiformis*; Sesb: *Sesbania rostrata*; Teph: *Tephrosia vogelii*. DF** indicates degree of freedom for both treatment and the experimental error.

within sampling dates. Counts were expressed as log(x+1) and percentages were arcsine-transformed before analyses in order to stabilise variances. The non-transformed means were reported, however. Means were separated with Student-Newman-Keuls (SNK) at p = 0.05.

Pearson's correlation analysis was used to examine whether pest numbers and damage affected crop yield and yield losses. Simple regression analyses were used to assess to what extent the numbers of each pest species accounted for ear damage and ear losses.

Results

Ear borer population densities in different intercrops

The results showed that intercropping maize with legumes or cassava has a significant effect on the infestation by *Mussidia nigrivenella* (F = 3.4, d.f. = (7; 712), p ≤ 0.015) and other ear borers including *Thaumatotibia leucotreta* (F = 2.5, d.f. = (7; 712), p < 0.05), but the performance of each treatment differed from one location to another. Significant interactions between cropping system and locations (p ≤ 0.02) showed that these factors jointly influenced the *M. nigrivenella* population. In Bantè, the intercrops significantly reduced the number of *M. nigrivenella* eggs and larvae and *Eldana saccharina* immatures compared to the monocrop (tab. 1), while only soybean, *Canavalia* and *Tephrosia* as companion crops showed significant effects on *Sesamia* spp. Overall, the ear borers recorded and numbers of larvae on maize cropped with *Sesbania* were similar to those in sole maize, and they were both significantly different from the rest of the treatments.

In Cana, a similar trend was observed on the number of *M. nigrivenella* eggs; the treatments were

Table 3. Effect of cropping systems on *Mussidia nigrivenella* and other maize ear borer infestations, ear weight and ear damages in Djidja, Benin

Intercrop	No. of M. nigrivenella eggs	No. of M. nigrivenella larvae+pupae	No. of Eldana saccharina	No. of Sesamia calamistis	No. of Other insects*	Overall ear borers	Ear weight (g)	% Ear damage	Ear loss (g)	% loss
Maize mono	0.00±0.00	0.27±0.08c	0.01±0.01	0.04±0.02	0.87±0.21	0.98±0.22c	119.05±8.69	7.56±2.13c	7.78±3.01c	6.55±1.93
Maize-Cass.	0.00±0.00	0.10±0.04a	0.00±0.00	0.01±0.01	0.37±0.08	0.48±0.15a	133.11±10.42	2.55±0.81ab	2.77±0.95ab	2.25±0.73
Maize-Cowp.	0.00±0.00	0.19±0.08bc	0.01±0.01	0.03±0.02	0.48±0.07	0.71±0.11b	126.84±8.02	3.29±1.51ab	3.31±1.08ab	2.94±1.36
Maize-Phaseo.	0.00±0.00	0.19±0.09bc	0.01±0.01	0.04±0.02	0.50±0.11	0.74±0.15b	125.82±5.76	4.42±1.82ab	3.89±1.83ab	3.51±1.31
Maize-Soyb.	0.00±0.00	0.12±0.05ab	0.01±0.01	0.02±0.02	0.47±0.10	0.62±0.13ab	130.38±11.57	3.18±0.85ab	3.20±0.83ab	2.71±0.73
Maize-Canav.	0.00±0.00	0.11±0.05a	0.00±0.00	0.02±0.02	0.45±0.12	0.58±0.12ab	135.85±7.52	2.21±0.69ab	2.59±0.83ab	1.94±0.61
Maize-Sesb.	0.00±0.00	0.20±0.09bc	0.01±0.01	0.04±0.02	0.68±0.17	0.84±0.20bc	122.39±9.58	6.66±3.77bc	6.38±3.19bc	5.64±3.06
Maize-Tephr.	0.00±0.00	0.08±0.03a	0.00±0.00	0.00±0.00	0.33±0.08	0.41±0.12a	138.45±9.25	1.20±0.51a	1.35±0.71a	1.04±0.46
DF**	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 232)	(7; 232)	(7; 232)	(7; 232)
P	1.00	0.031	0.885	0.470	0.064	0.05	0.087	0.050	0.050	0.027

Means within columns followed by the same letter are not significantly different (SNK, P=0.05). *Other insects are *Thaumatotibia leucotreta*, beetles (*Sitophilus zeamais, Carpophilus* sp. and *Cathartus quadricollis*). Cass: cassava; Cowp: cowpea; Phaseo: *Phaseolus lunatus*; Soyb: soybean; Canav: *Canavalia ensiformis*; Sesb: *Sesbania rostrata*; Teph: *Tephrosia vogelii*. DF** indicates degree of freedom for both treatment and the experimental error.

Table 4. Effect of cropping systems on *M. nigrivenella* and other maize ear borer infestations, ear weight and ear damages at IITA, Abomey-Calavi, Benin.

Intercrop	No of *M. nigrivenella* eggs	No of *M. nigrivenella* larvae+pupae	No of *E. saccharina*	No of *S. calamistis*	No of Other insects*	Total ear borers	Ear weight (g)	% Ear damage	Ear loss (g)	% loss
Maize mono	0.10±0.06b	0.54±0.10b	0.16±0.05c	0.30±0.06b	0.75±0.16b	1.56±0.21b	119.21±6.40a	13.50±2.53b	13.24±2.31b	11.72±2.17b
Maize-Cass.	0.00±0.00a	0.27±0.06ab	0.06±0.02ab	0.13±0.04a	0.53±0.08a	0.95±0.14a	139.07±5.94b	7.29±1.60a	8.77±1.57ab	6.58±1.19ab
Maize-Cowp.	0.00±0.00a	0.40±0.07ab	0.10±0.04b	0.18±0.04a	0.66±0.12ab	1.24±0.20ab	127.62±7.28ab	10.85±2.79ab	9.69±1.36ab	10.42±2.85ab
Maize-Phaseo.	0.00±0.00a	0.41±0.08b	0.12±0.06bc	0.19±0.08a	0.70±0.12b	1.32±0.17ab	125.89±8.68ab	11.18±1.75ab	10.30±1.89ab	9.55±1.45ab
Maize-Soyb.	0.00±0.00a	0.39±0.07a	0.09±0.04b	0.16±0.05a	0.57±0.10a	1.11±0.14a	133.35±9.61b	10.70±3.87a	8.88±2.38ab	9.27±3.23ab
Maize-Canav.	0.00±0.00a	0.32±0.07a	0.08±0.03ab	0.13±0.04a	0.55±0.09a	1.00±0.16a	136.53±6.86b	7.35±1.34a	8.51±1.22ab	6.26±0.90ab
Maize-Sesb.	0.00±0.00a	0.42±0.06a	0.12±0.05bc	0.28±0.12b	0.72±0.16	1.42±0.22b	117.10±6.73a	12.02±3.41b	12.21±2.68b	9.02±2.10b
Maize-Tephr.	0.00±0.00a	0.26±0.05a	0.04±0.02a	0.11±0.03a	0.47±0.11a	0.86±0.12a	141.95±8.26b	6.99±1.01a	6.95±1.47a	6.24±1.39a
DF**	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 230)	(7; 230)	(7; 230)	(7; 230)
P	0.046	0.034	0.002	0.043	0.062	0.045	0.045	0.048	0.043	0.053

Means within columns followed by the same letter are not significantly different (SNK, P = 0.05). *Other insects are *Thaumatotibia leucotreta*, beetles (*Sitophilus zeamais*, *Carpophilus* sp. and *Cathartus quadricollis*). Cass: cassava; Cowp: cowpea; Phaseo: *Phaseolus lunatus*; Soyb: soybean; Canav: *Canavalia ensiformis*; Sesb: *Sesbania rostrata*; Teph: *Tephrosia vogelii*. DF** indicates degree of freedom for both treatment and the experimental error.

not as effective as in Bantè in reducing *M. nigrivenella* larvae, except for maize intercropped with soybean and *Tephrosia* (tab. 2). The treatments that reduced *M. nigrivenella* infestation were not always effective against other insects found in the ear; for instance, the numbers of *E. saccharina* on maize intercropped with cowpea and *Phaseolus* were similar to those of the control. Overall, only maize intercropped with cassava,
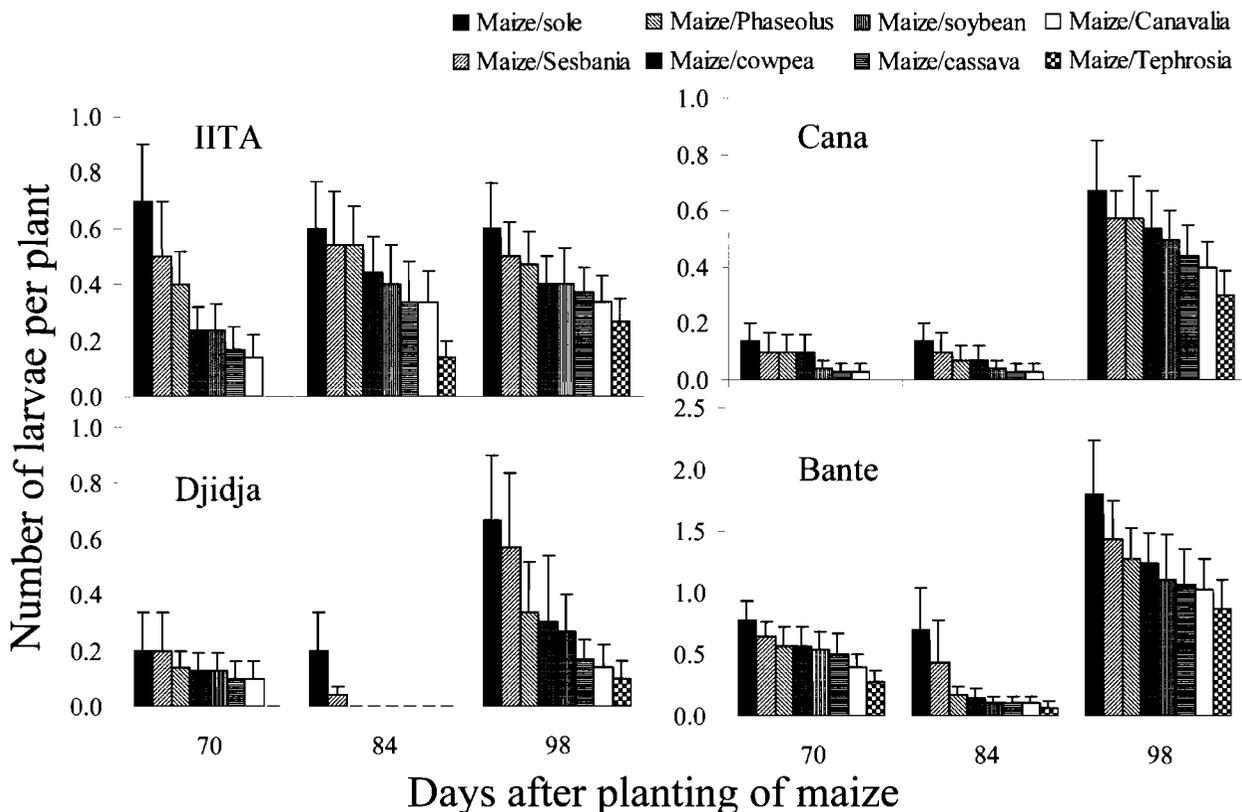
Figure 1
Numbers of *Mussidia nigrivenella* larvae collected per plant and per planting system in four locations during the three sampling dates in Benin.

soybean, *Canavalia* and *Tephrosia* gave a significant reduction in pest populations.

In Djidja, eggs were rarely found in any of the treatments. The numbers of *M. nigrivenella* larvae in the maize-*Canavalia* and -*Tephrosia* intercrops were significantly different from those in maize intercropped with cowpea, *Phaseolus* and *Sesbania*, which were similar to those in the monocrop (tab. 3). No difference was found in the infestation by *Eldana* spp., *Sesamia* spp. and other insects found in the ear between mono- and intercrops ($P \geq 0.05$). Overall, insect numbers in the ear were significantly affected by the intercrops ($P = 0.05$).

At IITA, eggs of *M. nigrivenella* were found in the mono- but rarely in the intercrops. Maize intercropped with soybean, *Canavalia* and *Tephrosia* reduced significantly the number of *M. nigrivenella* larvae compared to maize intercropped with *Phaseolus*, which was similar to that of the monocrop (tab. 4). Cassava and cowpea had intermediate effects. *Eldana* spp. infestations were also significantly reduced in the intercrops. The total ear borers were significantly reduced by cassava, soybean, *Canavalia* and *Tephrosia* in the system.

Although all intercrops had an effect on *M. nigrivenella* infestation, the combination of maize-*Canavalia* and maize-*Tephrosia* proved to be the most effective in the different locations. Few numbers of *M. nigrivenella* larvae were recorded on *Canavalia* and cowpea, where most larvae were *Maruca vitrata* Fabricius 1787 (Lepidoptera: Pyralidae).

No parasitoids were found on *M. nigrivenella* during the experiment in any of the locations. The data across locations showed that *M. nigrivenella* population densities varied significantly with time of sampling (fig. 1). More larvae and pupae were found during the last sampling when the maize was ready to be harvested.

Influence of intercrops on maize yield and on ear borer damages

Ear damage and grain losses in the different locations were significantly lower in the inter- than in the monocrop except for maize intercropped with *Sesbania*. Ear damage in intercrops was reduced by 34.3–61.1% in Bantè, 19.1–50.2% in Cana, 11.9–84.1% in Djidja and 11–48.2% at IITA compared to the maize monocrop (tabs.1–4). The percentage loss was higher in the monocrop and in maize intercropped with cassava, *Phaseolus*, cowpea and soybean than in maize intercropped with *Canavalia* and *Tephrosia*. Ear weight losses were reduced in the intercrops by 22.1–51% in Bantè, 0.9–80.5% in Cana, 13.9–84.1% in Djidja and 11.1–46.8% at IITA (tabs. 1–4). The highest reduction in ear damages and losses were found in maize intercropped with *Tephrosia*. However across locations, the intercrops generally had no effect on ear weight ($P > 0.05$). Across the four experimental locations, ear weight was negatively correlated with the number of ear borers (tab. 5), but ear damage increased with the number of insects found in the ear. Multiple regressions between ear damage and insect variables showed that the numbers of *M. nigrivenella*, *E. saccharina*, *S. calamistis* and other insects including *T. leucotreta*, significantly affected the percentage of ear damage and ear losses (tab. 6).

Discussion

As shown for stem borers by Schulthess *et al.* (2004) and Chabi-Olaye *et al.* (2005), this study has demonstrated that intercropping reduces attack of maize ears by *Mussidia nigrivenella* and other stem

Table 5. Pearson correlation coefficients between maize yield, pest and damage variables using data across the four experimental locations in Benin.

	1	2	3	4	5	6	7	8	9
1	1.00								
2	−0.31**	1.00							
3	−0.18**	0.92**	1.00						
4	−0.01	0.01	0.01	1.00					
5	−0.13**	0.51**	0.55**	0.03	1.00				
6	−0.12**	0.48**	0.41**	−0.01	0.13**	1.00			
7	−0.22**	0.68**	0.54**	−0.02	0.23**	0.16**	1.00		
8	−014**	0.54**	0.45**	−0.03	0.05*	0.12**	0.17**	1.00	
9	−0.17**	0.78**	0.75**	−0.01	0.60**	0.35**	0.48**	0.74**	1.00

*r values ≥ 0.04 have $P \leq 0.05$ and **r values ≥ 0.10 have $P < 0.01$.
1. Ear weight (g); 2. % Ear damage; 3. % Yield loss; 4. Number of *Mussidia nigrivenella* eggs; 5. Number of *M. nigrivenella* (larvae + pupae); 6. Number of *Eldana saccharina* larvae; 7. Number of *Sesamia calamistis*; 8. Number of other insects (*Thaumatotibia leucotreta* + Coleoptera larvae) in the ear; 9. Overall larvae in ear.

borer species that feed in the ear. Root (1973) and Andow (1991) have suggested that the herbivores are likely to find and remain on host plants that occur in large, dense and pure stands, due to the resource concentration factor. It also has been suggested that when diverse backgrounds 'disrupt' (Vandermeer 1989) insects from selecting otherwise-acceptable host plants, the action is mediated through, among other factors, visual camouflage (Smith 1969) or deterrent or repellent chemicals (Uvah & Coaker 1984). Ndemah and colleagues (2003) have suggested that the negative relationship between the non-host and plant density and the numbers of larvae are probably due to difficulties encountered by the female moths in finding host plants for oviposition.

Vandermeer (1989) listed three possible mechanisms responsible for reducing pest infestation in mixed cropping system: (i) the disruptive-crop hypothesis, in which a second non-host plant species disrupts the ability of the pest to attack its proper host plant species; (ii) the trap crop hypothesis, in which a second non-suitable host plant species attracts the pest away from its primary host; and (iii) the natural enemy hypothesis, in which the intercropping set-up attracts more predators and

parasitoids than the monocrop, thereby reducing pests on the primary host plant. In the present study, although each treatment had an effect on borer infestation, the most effective intercrops in the different locations were the treatments where maize was intercropped with *Canavalia* and *Tephrosia*.

According to Sétamou and co-workers (1999), jackbean (*C. ensiformis*) is the most suitable host plant for *M. nigrivenella* development. The high suitability of this cover crop for the pest's development and survival compared to maize might have direct effects on its population dynamics in maize. In our experiments, maize and jackbean seeds were sown simultaneously and both plants reached the stage suitable for *M. nigrivenella* attack at the same time; this could explain why low numbers of the pest were found in the maize-jackbean intercrop. The low number of *M. nigrivenella* observed in the maize-*Tephrosia* intercrop is probably due to the repulsive effect of *T. vogelii*. In a semi-field study, oviposition of *M. nigrivenella* was reduced by the leaf extract of *T. vogelii*, showing its activity as an oviposition deterrent (Agbodzavu 2005). These results suggest, moreover, that the attractiveness and deterrence of the legumes intercropped with maize further increase the

Table 6. Multiple regressions between damage and insect variables.

Variables	Coefficient ± SE	Partial T-value	Mean ± SE	Partial P
Dependent: arcsin√(% Ear damage)			10.64 ± 0.208	
Independent variables :				
$\log_{10}$ (No. of *Mussidia. nigrivenella*+1)	25.46 ± 1.43	17.80	0.614 ± 0.013	<0.0001
$\log_{10}$ (No. of *Eldana saccharina*+1)	30.89 ± 2.70	11.43	0.118 ± 0.006	<0.0001
$\log_{10}$ (No. of *Sesamia* sp. +1)	48.21 ± 2.62	18.40	0.192 ± 0.005	<0.0001
$\log_{10}$ (No. of other insects +1)	24.73 ± 1.07	23.02	1.124 ± 0.036	<0.0001
Intercept = 2.46 ± 0.39				
N= 954, F= 653.10, P< 0.0001, R^2= 0.73				
Dependent: arcsin√(% yield loss)			7.36 ± 0.139	
Independent variables:				
$\log_{10}$ (No. of *M. nigrivenella* +1)	21.76 ± 1.19	18.29	0.614 ± 0.013	<0.0001
$\log_{10}$ (No. of *E. saccharina* +1)	16.46 ± 2.26	7.28	0.118 ± 0.006	<0.0001
$\log_{10}$ (No. of *Sesamia* sp. +1)	32.95 ± 2.18	15.13	0.192 ± 0.005	<0.0001
$\log_{10}$ (No. of other insects +1)	15.14 ± 0.90	16.84	1.124 ± 0.036	<0.0001
Intercept =3.59 ± 0.33				
N= 942, F= 437.42, P< 0.0001, R^2= 0.65				
Dependent: g Ear loss			5.60 ± 0.099	
Independent variables:				
No. of *M. nigrivenella*	1.62 ± 0.22	7.31	0.614 ± 0.013	<0.0001
No. of *E. saccharina*	1.50 ± 0.45	3.30	0.118 ± 0.006	0.0010
No. of *Sesamia sp*	7.36 ± 0.65	11.30	0.192 ± 0.005	<0.0001
No. of borers in stem	1.88 ± 0.71	2.64	0.309 ± 0.009	0.0085
No. of other insects	0.28 ± 0.08	3.47	1.124 ± 0.036	0.0006
Intercept = 2.55 ± 0.29				
N= 942, F= 71.86, P< 0.0001, R^2=0.28				

effectiveness of intercropping in suppressing lepidopteran insects on maize ears. Visual and chemical stimuli from the host and non-host plants might also affect the rate at which insects colonise habitats, and their behaviour in those habitats. Moreover in an intercrop, the primary host plant is made less attractive to the herbivore, and this may depend on the kind of cues, either olfactory or tactile, perceived by the insect. Volatiles emanating from plant tissues have been reported as influencing attractiveness of the plant (Elzen *et al.* 1984; Udayagiri & Jones 1992), which may have also played a vital role in this experiment.

Although it is often assumed that intercropping may enhance the effectiveness of natural enemies, there was no support for this hypothesis in the present study. No parasitoids were found in any of the treatments, including the monocrop, in our study. According to Sétamou *et al.* (2002), natural enemies of *M. nigrivenella* are rare in cropping systems and wild habitats in Benin, suggesting that the reduction in pest infestation in this study was not due to parasitism but depended on the performance (attractiveness or repulsiveness) of each intercrop plant or ovipositional preference of the ear borer.

The effectiveness of the treatments differed from one location to another and with the borer species. Sétamou and colleagues (2000b), showed that the abundance of *M. nigrivenella* is more pronounced in the Northern Guinea Savanna than in the other regions under study, due to the abundance of its host plants. The differences observed in the treatments on infestation by *M. nigrivenella* and *Eldana saccharina* could be explained by the differences in the oviposition behaviour of the two borers. *E. saccharina*, which primarily is a stem borer that later moves into the ear (Schulthess *et al.* 1997), infests plant at the tasselling stage or later (Kaufman 1983), whereas the ear borer *M. nigrivenella* oviposits on the silk or husks of young and old ears; *M. nigrivenella* has been recorded on various plants including the legumes tested in this study (Sétamou *et al.* 2000a).

Our study has demonstrated that a change in the vegetation diversity could change the abundance and incidence of maize ear borers. The importance of intercropping as a method of controlling stem borers in sorghum and maize has been reported by Amoako-Atta & Omolo (1983), Ampong-Nyarko *et al.* (1994), Skovgard & Paets (1996) and Ayisi *et al.* (2001). Intercropping has been successfully used in reducing infestation of maize stem borers, especially *Busseola fusca* Fuller 1901 (Lepidoptera: Noctuidae) (Chabi Olaye *et al.* 2005) and *Chilo partellus* Swinhoe 1885 (Lepidoptera: Pyralidae) (Ampong-Nyarko *et al.* 1994; Maluleke *et al.* 2005). Maize-bean intercropping experiments conducted in Ethiopia during the 1992-cropping season showed that sole maize had a significantly higher incidence of

stem borers and earworms as compared to intercropped treatments (Nigussie & Reddy 1996). By contrast Schulthess *et al.* (2004) could not show any effect on the ear-boring pests such as *M. nigrivenella* and *T. leucotreta* by intercropping maize with cassava, perhaps because they planted the maize before cassava.

There was an increase in the incidence of infestation of *M. nigrivenella* at the last sampling date compared to the first one. The low numbers of larvae encountered during the first sampling might have been caused by high mortality of immatures and the high numbers of larvae at the last sampling may have been due to an accumulation of two generations of *M. nigrivenella* on maize from the milk stage till harvest. According to Sétamou *et al.* (1999), the generation time of *M. nigrivenella* on maize is 37.5 days and the pest continues to infest the ear from the milk stage till harvest and even in stores. This oviposition behaviour of *M. nigrivenella* in the field can explain the presence of larvae of all stages and pupae in the maize ear during the last sampling in the present study.

In contrast to expectations, the high incidence of borer infestation had little effect on the ear weight, an indication that the threshold level of the pest was not reached, although the grain loss was affected by the treatments. This may have a great impact on the aflatoxin content of the maize grains. A study conducted by Hell and co-workers (2003) has shown that association of grain legumes or groundnut with maize would increase the aflatoxin content in maize. In the present study, aflatoxin was not measured in the maize samples. In future, we recommend that in maize-legume intercropping studies, care should be taken to assess the aflatoxin content in each treatment before the best crop combinations, that will not only reduce the pest incidence but also aflatoxin contamination, are selected.

In conclusion, the findings of this study show that maize-legumes or maize-cassava intercrops can reduce *M. nigrivenella* and other ear borers, including *Thaumatotibia leucotreta* infestation, compared to maize monoculture. This study shows that an intercropping system with 'poor' hosts of *M. nigrivenella* could be developed in a 'push-pull' strategy for the control of *M. nigrivenella* in small-scale maize farming systems. This strategy will involve using *Canavalia ensiformis* as the highly susceptible trap plant (providing the 'pull') and *Tephrosia vogelii* as the repellent intercrop (the 'push'). Tests are being conducted to determine the susceptible stages of *C. ensiformis*, the most preferred host plant of the ear borer.

References

Agbodzavu K.M. 2005. *Evaluation de l'efficacité de quatre extraits* végétaux (Azadirachta indica, Jatropha curcas, Hyptis suaveolens, Tephrosia vogelii*) dans le contrôle de* Mussidia nigrivenella *(Lep. Pyralidae) au champ et au laboratoire.* Mémoire d'Ingénieur Agronome, Ecole Supérieure d'Agronomie, Université de Lomé, Togo, 85 p.

Amoako-Atta B., Omolo O. 1983. Yield losses caused by stem-pod-borer complex within maize-cowpea-sorghum intercropping systems in Kenya. *Insect Science and its Application* 4: 49-46.

Ampong-Nyarko K., Reddy K.V., Nyang'or R.A., Saxena K.N. 1994. Reduction of pest attack on sorghum and cowpea by intercropping. *Entomologia Experimentalis et Applicata* 70: 179-184.

Andow D.A. 1991. Vegetational diversity and arthropod population response. *Annual Review of Entomology* 36: 261-586.

Ayisi K.K., Mposi M.S., van den Berg J. 2001. Grain yield response and Chilo partellus infestation in diverse sorghum: cowpea intercrop arrangements. *South African Journal of Plant Soil* 18: 39-41.

Bosque-Pérez N.A., Mareck J.H. 1990. Distribution and species composition of lepidopterous maize borers in Southern Nigeria. *Bulletin of Entomological Research* 80: 363-368.

Carsky R.J., Douthwaite B., Manyong V.M., Sanginga N., Schultz S., Vanlauwe B., Diels J., Keatinge J.D.H. 2003. Amélioration de la gestion des sols par l'introduction de légumineuses dans les systèmes céréaliers des savanes africaines. *Cahiers Agriculteurs* 12: 227-233.

Chabi-Olaye A. , Nolte C., Schulthess F., Borgemeister C. 2005. Relationships of intercropped maize, stem borer damage to maize yield and land-use efficiency in the humid forest of Cameroon. *Bulletin of Entomological Research* 95: 417-427.

Dissemond A., Hindorf H. 1990. Influence of sorghum-maize-cowpea intercropping on the insect situation at Mbita-Kenya. *Journal of Applied Entomology* 109: 144-150.

Elzen G.W., Williams H.J., Vinson S.B. 1984. Isolation and identification of cotton synomones mediating searching behavior by parasitoid Campoletis sonorensis. *Journal of Chemical Ecology* 10: 1251-1264.

Hell K., Cardwell K.F., Poehling H.-M. 2003. Relationship between management practices, fungal infection and aflatoxin for stored maize in Benin. *Journal of Phytopathology* 151: 690-698.

Kaufman T. 1983. Behavioral biology, feeding habits and ecology of three species of maize stem borers: Eldana saccharina (Lepidoptera: Pyralidae), Sesamia calamistis and Busseola fusca (Noctuidae) in Ibadan, Nigeria, West Africa. *Journal of the Georgia Entomological Society* 18: 259-272.

Maluleke M.H., Addo-Bediako A., Kingsley K.A. 2005. Influence of maize-lablab intercropping on the lepidopterous stem borer infestation in maize. *Journal of Economic Entomology* 98: 384-388.

Moyal P. 1988. *Les foreurs du maïs en zone des savanes en Côte d'Ivoire. Données morphologiques, biologiques, écologiques, essais de lutte et relation plante-insecte.* ORSTOM, Paris, collection Etudes et Thèses, 367 p.

Moyal P. 1993a. Maize crop intensification and borer attacks in the Ivory Coast: insect population, p. 253-265 *in:* **Paoletti M. G., Foissner W., Coleman D. (eds.),** *Soil Biota, Nutrient Cycling and Farming Systems.* Lewis Publishers, Boca Raton.

Moyal P. 1993b. Maize crop intensification and borer attacks in the Ivory Coast: yields, p. 267-278 *in:* **Paoletti M. G., Foissner W., Coleman D. (eds.),** *Soil Biota, Nutrient Cycling and Farming Systems.* Lewis Publishers, Boca Raton.

Moyal P., Tran M. 1991a. Ear borer Mussidia nigrivenella (Lepidoptera: Pyralidae) of maize in Ivory Cost. I-Morphological and biological data. *Insect Science and its Application* 12: 209-214.

Moyal P., Tran M. 1991b. Ear borer Mussidia nigrivenella (Lepidoptera: Pyralidae) of maize in Ivory Cost. II-Ecological data. *Insect Science and its Application* 12: 215-223.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Poehling M., Cardwell K.F. 2003. Factors affecting infestations of the stalk borer Busseola fusca

(Fuller) on maize in the forest zone of Cameroon with special reference to scelionid egg parasitoids. *Environmental Entomology* 32: 61-70.

Nigussie T., Reddy M.S. 1996. Performance of maize-bean intercropping systems under low and medium rainfall situations: insect pests incidence, *in:* **Eshetu B., Abdurahman A., Aynekulu Y. (eds.),** *Proceedings of the Third Annual Conference of the Crop Protection Society of Ethiopia, 18-19 May 1995.* CPSE, Addis Ababa.

Oloo G.W., Ogeda O. 1990. The role of local natural enemies in population dynamics of Chilo partellus (Swinh) under subsistence farming system in Kenya. *Insect Science and its Application* 10: 243-251.

Omolo E.O. 1986. Stem borer incidence in sorghum intercropped with maize and cowpea tested in Kenya. Sorghum and millet improvement in eastern Africa, p367-378 *in: Proceedings of the 4th regional workshop,* Soroti, Uganda, Nairobi, Kenya, SAFGRAD-ICRISAT.

Paets P., Ekbom B., Skovgard H. 1997. Influence of intercropping on the abundance, distribution and parasitism of Chilo spp. (Lepidoptera: Pyralidae) eggs. *Bulletin of Entomological Research* 87: 507-513.

Root R.B. 1973. Organization of plant-arthropod association in simple and diverse habitats: the fauna of collards (Brassica oleracea). *Ecological Monographs* 43: 95-124.

SAS Institute 1997. *SAS-STAT software: changes and enhancements. Release 6.12,* SAS Institute Inc., Cary, North Carolina, 1167 p.

Schulthess F., Bosque-Pérez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Georgen G. 1997. Exchanging natural enemies species of lepidopterous cereal stem borers between African regions. *Insect Science and its Application* 17: 97-108.

Schulthess F., Chabi-Olaye A., Gounou S. 2004. Multi-trophic level interactions in a cassava-maize mixed cropping system in the humid tropics of West Africa. *Bulletin of Entomological Research.* 94: 261-271.

Sétamou M., Schulthess F., Bosque-Pérez N.A., Poehling H-M., Borgemeister C. 1999. Bionomics of Mussidia nigrivenella (Lepidoptera: Pyralidae) on three host plants. *Bulletin of Entomological Research* 89(5): 465-472.

Sétamou M., Schulthess F., Poehling H.-M., Borgemeister C. 2000a. Host plants and populations dynamics of the ear borer Mussidia nigrivenella Ragonot (Lepidoptera: Pyralidae) in Benin. *Journal of Environmental Entomology* 29: 516-524.

Sétamou M., Schulthess F., Poehling H-M., Borgemeister C. 2000b. Monitoring and modeling of field infestation and damage by the maize ear borer Mussidia nigrivenella (Lepidoptera: Pyralidae) in Benin, West Africa. *Journal of Economic Entomology* 93(3): 650-657.

Sétamou M., Schulthess F., Goergen G., Poehling H-M., Borgemeister C. 2002. Natural enemies of the maize ear borer, Mussidia nigrivenella Ragonot (Lepidoptera: Pyralidae) in Benin, West Africa. *Bulletin of Entomological Research* 92: 343-349.

Shanower T.F., Schulthess F., Gounou S. 1991. *Distribution and abundance of some stem and ear borers in Benin.* Plant Health Management Research Monograph 1, International Institute of Tropical Agriculture, Ibadan, Nigeria, 18 p.

Silvie P. 1993. Nouvelles données sur Mussidia nigrivenella Ragonot (Lepidoptera: Pyralidae) au Togo. *Insect Science and its Application* 14: 643-649.

Skovgard H., Paets P. 1996. Effects of intercropping on maize stem borers and their natural enemies. *Bulletin of Entomological Research* 86: 599-607.

Smith J.G. 1969. Some effects of crop background on the populations of aphids and their natural enemies on Brussels sprouts. *Annals of Applied Biology* 63: 326-330.

Udayagiri S., Jones R.L. 1992. Flight behavior of Macrocentrus grandii Goidanich (Hymenoptera: Braconidae), a specialist parasitoid of European corn borer (Lepidoptera: Pyralidae): factors influencing response to corn volatiles. *Environmental Entomology* 21: 1448-1456.

Uvah I.I.I., Coaker T.H. 1984. Effect of mixed cropping on some insect pests of carrots and onions. *Entomologia Experimentalis et Applicata* 36: 159-167.

Vandermeer J. 1989. *The Ecology of intercropping.* Cambridge University Press, Cambridge, 237 p.

Assessment of the impact of natural enemies on stemborer infestations and yield loss in maize using selected insecticides in Mozambique

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Abstract. The effect of natural enemies on stemborer infestations and maize grain yields was estimated using an insecticide exclusion method. Field experiments were conducted at low, mid and high elevation zones, which vary in the stemborer species composition. Dimethoate was applied to exclude natural enemies and Cypermethrin to suppress stemborers, while other plots served as control. At all study sites more stemborer larvae and pupae were collected when natural enemies were excluded. Parasitism as well as maize grain weight in the unprotected plots were significantly higher than in the exclusion plots. Yield losses increased by 28.9 % in unprotected to 43.3 % in exclusion plots. The most abundant parasitoids of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) were *Cotesia sesamiae* (Cameron), *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) and *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae). While for *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) they were *C. sesamiae*, *Sturmiopsis parasitica* (Curran) (Diptera: Tachinidae) and *Porcerochasmias nigromaculatus* Heinrich (Hymenoptera: Ichneumonidae). It was concluded that exclusion of natural enemies caused an increase in stemborer populations, thus, the parasitoids play an important role in suppressing stemborer infestations and increase maize yield.

Résumé. Estimation de l'impact des ennemis naturels sur l'infestation par les foreurs et les pertes de rendement du maïs au Mozambique en utilisant des insecticides. Les effets d'ennemis naturels sur des foreurs qui infestent du maïs ainsi que sur le rendement de ce dernier ont été estimés par une méthode d'exclusion à l'aide d'insecticides. Les expérimentations ont été menées dans des champs à basse, moyenne et haute altitude, infestés par des espèces différentes de foreurs. Les ennemis naturels ont été éliminés à l'aide de diméthoate et de cyperméthrine. Dans tous les sites étudiés, une quantité plus importante de larves et de chrysalides a été collectée après élimination des ennemis naturels. Le parasitisme ainsi que le poids des grains de maïs ont été significativement plus élevés dans les parcelles non traitées par insecticides (parcelles témoins) que dans les traitées. La perte de rendement a augmenté de 28.9 % dans les parcelles témoins et de 43.3 % dans celles traitées. Les espèces de parasitoïde de *Chilo partellus* (Swinhoe) (Lepidoptera : Crambidae) les plus abondantes étaient *Cotesia sesamiae* (Cameron), *Cotesia flavipes* Cameron (Hymenoptera : Braconidae) et *Dentichasmias busseolae* Heinrich (Hymenoptera : Ichneumonidae) ; alors que celles de *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae) étaient *C. sesamiae*, *Sturmiopsis parasitica* (Curran) (Diptera : Tachinidae) et *Porcerochasmias nigromaculatus* Heinrich (Hymenoptera : Ichneumonidae). On en a tiré la conclusion que l'élimination des ennemis naturels des foreurs a causé une augmentation de la population de ces derniers, et par conséquent que les parasitoïdes jouent un rôle important dans la régulation des infestations de foreurs et dans l'augmentation du rendement du maïs.

Keywords: Stemborer, *Chilo partellus*, *Busseola fusca*, natural enemies, exclusion, maize yield loss.

Lepidopteran stemborers such as the invasive *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and the indigenous *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) are the most important pests of maize and grain sorghum in Mozambique. The pink stemborer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), which is of minor importance (Gonçalves 1970;

Segeren *et al.* 1991), is usually kept under control by its natural enemies (Kfir 1998).

Frequently, up to 100% of plants are infested in southern Mozambique, where *C. partellus* is the most abundant species (Berger 1981; Cugala *et al.* 2001). Crop losses of between 50 and 100% in small-scale farmers' fields have been reported (Segeren *et al.* 1991).

A wide range of egg, larval and pupal parasitoids has been recorded from maize and sorghum in Mozambique (Gonçalves 1970; Segeren *et al.* 1991; Davies *et al.* 1995; Cugala *et al.* 1999; 2001; Cugala

& Omwega 2001). Among them, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) is the most important mortality factor of stemborer larvae. In addition, Gonçalves (1970) recorded 60% parasitism of *C. partellus* eggs due to *Trichogramma sp.* While Davies *et al.* (1995) recorded 20% parasitism of *B. fusca* larvae due to *C. sesamiae*. The exotic larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was released for the first time in 1996 in the Southern and in 1999 in the Central regions of Mozambique. The parasitoid became established at the majority of release sites and it is spreading to new areas where it has not been released (Cugala *et al.* 1999; 2001; Cugala & Omwega 2001). Levels of parasitism of up to 40% due to *C. flavipes* have been reported on *C. partellus* at one of the release sites (Cugala *in lit.*).

In South Africa, Kfir (2002) reported high infestations by stemborers on sorghum due to partial elimination of parasitoids by applying pesticides. The present study attempts to assess to which extent yield losses due to stemborers are prevented by natural enemies by excluding them with insecticides.

Materials and methods

Study sites

Trials were conducted in three agroecological zones. The lowland location was Chokwe village (24°29'58"S; 32°57'57" E, elevation 80 m), a warm area in the southern province of Gaza, where *C. partellus* was the dominant stemborer (>90%), followed by *S. calamistis* (<10%) (Segeren *et al.* 1991; Cugala *et al.* 1999; 2001). *B. fusca* was not recorded from this area. The second area was Machipanda (18°52'16"S; 32°47'96"E, elevation 800 m), a medium to high elevation zone in the Central Province of Manica. In Machipanda, *B. fusca* represented 32% of the total borer population, while *C. partellus* represented 61% (Cugala *et al.* 2001). The third and high altitude location was Lichinga (>1000 m asl), where *B. fusca* was the dominant species (>90%), followed by *S. calamistis*.

Experimental design

A plot of 50 × 50 m² was prepared at each study site. The plot was divided in 12 subplots of 10 × 10 m² each with a 2 and 5 m space between plots and blocks respectively. A randomised complete block design (RCBD) with three treatments replicated four times was used. The treatments were 1) a control without insecticide application, 2) application of Dimethoate at a rate of 0.5 ml/l of water, which is 1/4 of the recommended dose of 2 ml/l of water, to exclude natural enemies as shown by Kfir (2002) and 3) a Cypermethrin treatment at 20 ml of insecticide/20 l of water to suppress both natural enemies and stemborers. These treatments will be referred to as unprotected, exclusion and fully protected, respectively. To ensure stemborer infestation, maize was planted in early January 2003 and 2004 at each site to coincide with the peak of stemborer infestation (February-March) and with a susceptible crop stage. Maize was planted at 90 cm between and 45 cm within row. NPK (12:24:12) at 200 Kg/ha was applied as basic fertilizer at planting and ammonium

nitrate (46% N) at 100 Kg/ha as top-dressing about four weeks after emergence. Chemical insecticides were applied according to a predetermined schedule at 15, 30, 45 and 60 days after crop emergence (DAE) as recommended by Segeren *et al.* (1991).

Stemborer abundance and parasitism

All plots were monitored at tasselling to evaluate numbers of larvae and pupae as well as associated parasitoids, and at harvest to determine yield losses due to stemborers.

To assess densities of larvae and pupae, 10 plants were randomly sampled from each plot and dissected. All larvae and pupae were placed individually in vials, taken to the laboratory and reared until adult or parasitoid emergence. The parasitoids were counted according to species. At tasseling, plant growth variables such as plant height, stem diameter and number of internodes, and damage variables such as number of internodes bored, tunnel length, number of holes and grain damage were recorded from each plot and at each study site.

Yield and yield losses

The harvested grain was sun-dried until the moisture content was between 13 and 14% for grain yield determination. The grain was then weighed and mean grain yield of each treatment calculated. Yield losses due to stemborer attack were estimated as differences between yield from the fully protected plots and yield from unprotected and exclusion plots according to the method described by Ampofo (1988) and expressed as a percentage of potential yield in fully protected plots:

$$Yp = (Yfp - Yu)/Yfp * 100$$

where Yp = yield loss in the presence of parasitoids, Yfp = yield of fully protected plots, Yu = yield of unprotected plots.

The difference between yields from fully protected plots and exclusion plots indicated the yield losses as a result of natural enemies' exclusion:

$$Ya = (Yfp - Yex)/Yfp * 100$$

where Ya = yield loss in the absence of parasitoids, Yex – yield of exclusion plots.

The impact of natural enemies (INE %) was estimated as the difference between the yield from unprotected and exclusion plots:

$$INE (\%) = (Yu - Yex)/Yu * 100$$

Data analysis

Data were subjected to analysis of variance (ANOVA) (Proc GLM; SAS Institute 1999) individually for each location. Means were separated using the Student Newman Keuls multiple range test when ANOVA was significant at $P < 0.05$. Relationships between the plant growth and damage variables, stemborers and parasitism levels were analysed using correlation and regression analysis (PROC CORR and REG; SAS). Number of insects and proportions were square root and arcsine of square root transformed, respectively, to normalize data before analysis.

Table 1. Effect of treatment on stemborer density and parasitism at the three study sites (±SE).

Location/ Treatment	% plants infested	# Stem- borer	C. flavipes	C. sesamiae	S. parasitica	D. busseolae	P. nigro-maculatus
a) *Chokwe*							
Unprotected	75±0.1b	2.9±2.3b	5.8±2.1a	13.2±4.2a	-	36.7±5.2a	-
Exclusion	90±1.2a	5.8±3.2a	0.0±0.0b	0.9±1.4b	-	10.3±2.4b	-
Fully protected	15±0.2c	0.9±0.8c	0.0±0.0b	0.0±0.0c	-	0.0±0.0	-
Df	2, 119	2, 119	2, 119	2, 119		2, 119	
F	45.5	22.0	3.3	34.6		21.8	
P-values	<0.0001	0.0003	0.0474	<0.0001		<0.0001	
b) *Machipanda*							
Unprotected	45±0.4b	3.4±2.2b	15.4±3.1a	20.4±4.4a	7.2±3.9a	20.9±2.4a	0.0±0.0
Exclusion	60±0.8a	6.8±3.2a	2.2±1.3b	5.1±2.1b	1.5±0.4b	4.0±3.2b	0.0±0.0
Fully protected	5±0.0c	0.5±0.8c	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.0±0.0	0.0±0.0
Df	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	
F	47.2	27.9	16.9	30.3	8.7	10.5	
P-values	<0.0001	<0.0001	<0.0001	<0.0001	0.0078	<0.0001	
c) *Lichinga*							
Unprotected	80±1.2a	4.8±2.4b	0.0±0.0	3.6±1.5a	4.5±1.3a	0.0±0.0	48.6±10.4a
Exclusion	95.5±1.5a	7.8±3.1a	0.0±0.0	0.0±0.0a	1.2±0.9a	0.0±0.0	2.6±1.2b
Fully protected	10±0.1b	1.2±1.6c	0.0±0.0	0.0±0.0a	0.0±0.0b	0.0±0.0	0.0±0.0c
Df	2, 119	2, 119		2, 119	2, 119		2, 119
F	56.0	78.8		5.1	8.3		27.0
P-values	<0.0001	<0.0001		0.0479	0.0004		0.0002

Means followed by same lowercase letter within column are not significantly different at *P* < 0.05 (SNK).

Results

Stemborers and parasitism levels

C. partellus was almost the only stemborer species collected in the lowland area at Chokwe. At Machipanda, *C. partellus* and *B. fusca* accounted for 68.3% and 31.7%, respectively, of the total stemborer population, while at Lichinga, it was 93.3% *B. fusca* and 6.7% *C. partellus*. Overall, numbers of *S. calamistis* were very low at all sites and, thus, were excluded from the data analyses.

In all three sites, percentage of infested plants and stemborer populations were highest in the exclusion and lowest in the fully protected plots (tab. 1).

The parasitoids collected at the study sites included the larval parasitoids *C. flavipes*, *C. sesamiae* and *Sturmiopsis parasitica* (Curran) (Diptera: Tachinidae), and the pupal parasitoids *Dentichasmias busseolae* Heinrich, *Procerochasmias nigromaculatus* Heinrich (Hymenoptera: Ichneumonidae) and *Pediobius furvus* Gahan (Hymenoptera: Eulophidae). Table 1 shows the percent parasitism by each parasitoid species. At Chokwe and Machipanda, *C. sesamiae* and *D. busseolae* were the most abundant larval and pupal parasitoids

of *C. partellus*, while in Lichinga the pupal parasitoid *P. nigromaculatus* was the most abundant on *B. fusca* followed by the larval parasitoid *S. parasitica*. In general, parasitoids and levels of parasitism were significantly more abundant on unprotected plots than in exclusion and fully protected plots at all study sites (P < 0.05) (tab. 1).

For the first time, the exotic parasitoid *C. flavipes* was recovered at Chokwe where it was released in 1998. At Chokwe and Machipanda, there were significant differences in parasitism due to *C. flavipes* and *C. sesamiae* between the unprotected and exclusion plots. The same pattern was observed for the pupal parasitoids *D. busseolae* and *P. nigromaculatus*. (tab. 1).

Damage symptoms and yield

In general, plant height, stem diameter and number of internodes were greater in fully protected and lowest in exclusion plots (tab. 2). For all damage variables and across sites, damage was greater in the unprotected and exclusion than the fully protected treatments and often higher in the exclusion than the unprotected plots (tab. 2).

There were no significant differences in cob weight between unprotected and exclusion plots at the three study sites (tab. 3). However, significantly higher cob weights were recorded in fully protected plots compared to the other treatments.

Significantly higher grain weights were obtained from fully protected plots compared to unprotected and exclusion plots at all sites ($P = 0.05$) (tab. 3).

When comparing grain weight from fully protected plots with that of unprotected plots, the grain weight loss varied between 28.9% and 34.5%. While when fully protected and exclusion plots were compared, the losses were between 36.4 and 43.3% (tab. 3). The percent differences between unprotected and exclusion plots estimated the impact of natural enemies on the yield of maize grain. It varied from 7.6 and 26.1% (tab. 3).

Relationships between plant growth, stemborers damage and grain weight

Significantly positive correlations were found between grain weight and plant growth variables such as plant height, stem diameter and number of internodes. By contrast, grain weight was significantly negatively correlated with proportion of internodes and number of holes bored, tunnel length, cob damage and number of stemborers per infested plant (tab. 4). Borer abundance was positively correlated with damage variables. Parasitism levels were positively associated with grain weight, proportion of internodes bored and number of holes.

For each site, the multiple regression analysis indicated a positive effect of plant height (Pht) and percent parasitism (Pab) on grain weight (Gw). On the other hand, the proportion of internodes bored (Pinod) and numbers of stemborers (Stb) had a negative effect on the grain weight (GW).

1. Chokwe:

$$Gw = -10.9 + 9.5Pht - 7.8Pinod - 1.9Stb + 1.7Pab$$
(P=0.0450, N=12, r^2=0.9190)

2. Machipanda:

$$Gw = 14.2 + 12.2Pht - 0.6Pinod - 1.1Stb + 5.9Pab$$
(P=0.0467, N=12, r^2=0.7314)

3. Lichinga:

$$Gw = 21.7 + 2.9Pht - 22.3Pinod - 0.4Stb + 0.6Pab$$
(P=0.0027, N=12, r^2=0.9613)

Table 2. Effect of different treatments on plant growth and damage variables at the three study sites (±SE).

Location/ Treatment	Plant height	Stem diameter	Number internodes	Proportion internodes bored	Stemborer holes	Tunnel length (cm)	Proportion plant tunnelled	Percent cob damage
a) *Chokwe*								
Unprotected	1.95±0.5b	2.4±0.2a	11.9±1.9b	0.57±0.2b	8.8±5.5a	50.6±23.5b	0.28±0.2b	14.3±22.7b
Exclusion	1.69±0.4c	2.1±0.6b	11.6±2.3b	0.78±0.1a	9.9±4.4a	69.8±39.3a	0.45±0.3a	54.8±29.8a
Fully protected	2.47±0.3a	2.5±0.3a	13.6±1.8a	0.05±0.1c	0.8±1.3b	3.9±6.8c	0.05±0.0c	5.1±12.8b
Df	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119
F	35.9	7.8	10.4	200.9	56.8	64.1	46.5	51.3
P-values	<0.0001	0.0007	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
b) *Machipanda*								
Unprotected	2.82±0.4a	1.95±0.3b	10.8±1.4a	0.45±0.27b	4.6±2.8a	20.8±11.2b	0.11±2.3a	25.6±2.8a
Exclusion	1.58±0.5b	1.84±0.3b	9.8±1.6b	0.65±0.2a	3.9±2.1b	31.5±19.1a	0.23±0.1a	33.3±28.1a
Fully protected	3.10±0.3a	2.30±0.2a	11.4±1.8a	0.04±0.1c	0.25±0.5c	2.15±3.3c	0.01±0.0b	0.0±0.0b
Df	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119
F	18.3	22.9	9.4	156.6	51.9	49.5	1.2	27.2
P-values	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.2972	<0.0001
c) *Lichinga*								
Unprotected	2.22±0.5b	1.83±0.2a	12.1±1.9a	0.53±0.2a	8.1±5.6a	28.7±20.7a	0.14±0.1b	22.7±25.6ab
Exclusion	1.54±0.2c	1.57±0.3b	9.4±1.8b	0.57±0.2a	9.0±5.6a	32.1±18.7a	0.21±0.1a	31.1±34.5a
Fully protected	2.8±0.4a	1.92±0.2a	12.5±1.9a	0.06±0.1b	0.8±1.4b	2.6±3.6b	0.01±0.0c	10.6±22.7b
Df	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119
F	98.5	18.4	31.4	113.4	41.5	39.5	41.6	5.1
P-values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0079

Means followed by same lowercase letter within column are not significantly different at P < 0.05 (SNK).

Discussion

The relative importance of stemborers in the present study corroborates results by Berger (1981), Segeren *et al.* (1991), Davies *et al.* (1995) and Cugala *et al.* (1999; 2001). The higher stemborer densities in the exclusion plots resulted in significantly higher plant damage, which negatively affected yields. Similarly, many authors reported a negative effect of mainly stem tunnelling, and, to a lesser extent, pest densities, on yield (Gounou *et al.* 1994; Ndemah *et al.* 2000; Ndemah & Schulthess 2002; Chabi-Olaye *et al.* 2005). High stemborer density in exclusion plots and subsequent plant damage was also observed by Kfir (2002), Kumar (1997) and Seshu Reddy & Sum (1992). Kfir (2002) working in South Africa estimated that the Dimethoate-treated plants were nearly two to three times more likely to be infested with stemborers than the unsprayed plants suggesting that a partial removal of natural enemies increased stemborer population, which agrees with findings in the present study. Furthermore, as in the present study, the chance of stemborers to be parasitized in the sprayed plots was 4 to 6 times less likely than in

unsprayed plots (Kfir 2002). Similar observations were made by Lim (1970) in Malaysia who reported an increase in damage score by stemborers in insecticide treated rice. Also, Eveleens *et al.* (1973) and Ehler *et al.* (1973) reported, that in cotton fields, Dimethoate suppressed predaceous insects but caused no harm to lepidopteran pests. Sinha *et al.* (1990) revealed that by topical application, Dimethoate was less toxic to first instar larvae of *Pieris brassica* L. (Lepidoptera: Pieridae) than seven other commonly used insecticides. Also Dimethoate was found to be more toxic to lepidopteran pests when ingested than by topical application, because Dimethoate is not readily absorbed by the lipids in the cuticle but can penetrate faster through the gut wall (Khan 1993). A recent screening of insecticides in Kenya and Tanzania, Dimethoate caused less than 20% mortality in the tetranychid mite *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), a key pest on tomato (M. Knapp, ICIPE, Nairobi, Kenya *pers. com.*), indicating resistance of the mite to the pesticide. In view of the present findings, Dimethoate should not be used without first determining its efficiency as it may aggravate pest infestations.

Table 3. Effect of treatments on cob and grain weight and yield losses at presence and absence of natural enemies (±SE).

Location/ Treatment	Cob weight Kg/plot	Grain weight kg/plot	Grain Yield losses from	
			Unprotected plots*	Exclusion plots**
a) *Chokwe*				
Unprotected	18.6±2.8b	17.1±1.5b	-	26.1 [a]
Exclusion	17.5±3.3b	12.6±1.48c	-	-
Fully protected	26.1±3.5a	22.3±1.0a	28.9	43.3 [b]
Df	2, 12	2, 12		
F	8.2	52.9		
P-values	0.0093	<0.0001		
b) *Machipanda*				
Unprotected	16.6±2.2b	14.1±2.6b	-	11.2 [a]
Exclusion	16.2±5.3b	12.9±5.5b	-	-
Fully protected	28.1±1.4a	21.4±3.4a	34.5	40.8 [b]
Df	2, 12	2, 12		
F	15.6	5.4		
P-values	0.0012	0.0290		
c) *Lichinga*				
Unprotected	18.2±1.70b	14.5±2.19b		7.6 [a]
Exclusion	17.6±0.91b	13.4±1.00b		
Fully protected	25.3±2.32a	21.1±2.01a	31.2	36.4 [b]
Df	2, 12	2, 12		
F	24.1	21.1		
P-values	0.0002	0.0004		

* = Grain yield losses in the presence of natural enemies (comparing fully protected and unprotected plots); ** = yield losses in the absence of natural enemies (comparing [a] unprotected and exclusion plots and [b] fully protected and exclusion plots).

In the present study, yield loss varied according to agro-ecological zone. Previous studies by Segeren *et al.* (1991) reported yield losses of 20% on-station and 40% on-farm. At all the three study sites, yield losses due to stemborer infestation were higher in the plots where natural enemies were excluded. The difference in stemborer damage and its impact on grain weight may be due to the differences in the stemborer species composition and their relative abundance between the three study sites. While *C. partellus* was the dominant borer in Chokwe, *B. fusca* was the most abundant in Lichinga. Both *C. partellus* and *B. fusca* were found coexisting in the same area and/or plant at Machipanda. Van den Berg *et al.* (1991) reported that *B. fusca* caused less stem damage and yield loss than *C. partellus*, both when occurring singly or in mixed populations with *C. partellus*. Thus, higher stemborer damage and yield losses are more likely to occur in areas where *C. partellus* is the abundant species compared to others areas where *B. fusca* is abundant. Ndemah & Schulthess (2002), using multiple regression of yield on numbers of individual borers species, argued that the higher damage caused by an individual *B. fusca* compared to *S. calamistis* and *E. saccharina* was due the larger size of larvae. However, immatures of *C. partellus* are considerably smaller than those of *B. fusca* (Muturi *et al.* 2006). It is suggested that the differences in yield losses caused by the two borer species might have been due to differences in the migration patterns of the larvae, duration of larval development and, thus, number of generation per season produced by the two stemborer species. For example, van den Berg *et al.* (1991) found that *C. partellus* migrates faster than *B. fusca*, thereby the same cohort of larvae may attack a higher number of plants.

Previous work indicated that despite the large numbers of parasitoids and relatively high parasitism levels by indigenous parasitoids of maize stemborers (Berger 1981; Gonçalves 1970; Kfir 1995), the parasitoids are not able to prevent economic damage and/or reduce pest populations to below economic threshold levels (Kfir 1997; 2000; Overholt 1998; Overholt *et al.* 1994). The present work, however, suggests that their impact on pest infestations and maize yield can be considerable. Kfir (1995) suggested that the low numbers of *S. calamistis* in South Africa were due to the action of its indigenous natural enemies and mainly *C. sesamiae*, which was also the most common larval parasitoid in the present study. By contrast, in West Africa, *C. sesamiae* is exceedingly rare and *S. calamistis* is one of the key pests of maize in the sub-humid tropics (Schulthess *et al.*, 1997). Similarly, in western Africa, there were hardly any studies on egg parasitoids of cereal stemborers until the 90s. However, more recent studies show that egg parasitism can be considerable (regional means of 95%) and parasitism early in the season was always positively related to maize yields at harvest (Sétamou & Schulthess 1995; Schulthess *et al.* 2001; Ndemah *et al.* 2003). Generally, studies on the role of indigenous parasitoids in controlling pests are scarce and it can be expected that their impact is underestimated. Furthermore, as suggested by Schulthess *et al.* (1997), because maize is not always present in the field and because of its high susceptibility to borer attacks, biological control has to come from wild habitats. In Benin, for example, parasitism by *S. parasitica* in maize stems, artificially infested with 3rd instar larvae of *S. calamistis*, were up 60%, versus less than 5% on naturally infested maize (Schulthess *in lit.*). Hence,

Table 4. Relationships between plant parameters, damage, parasitism and yield.

	1	2	3	4	5	6	7	8	9	10
1.	1.00									
2.	0.90*	1.00								
3.	0.53*	0.58*	1.00							
4.	0.40*	0.43*	0.28	1.00						
5.	0.43*	0.48*	0.72*	0.60*	1.00					
6.	−0.77*	−0.75*	−0.73*	−0.37*	−0.50*	1.00				
7.	−0.64*	−0.61*	−0.53*	−0.25	−0.27	0.78*	1.00			
8.	−0.51*	−0.51*	−0.62*	−0.10	−0.27	0.83*	0.79*	1.00		
9.	−0.54*	−0.56*	−0.54*	−043*	−0.40*	0.70*	0.56*	0.65*	1.00	
10.	−0.65*	−0.69*	−0.77*	−0.55*	−0.61*	0.76*	0.62*	0.62*	0.60*	1.00
11.	0.50*	0.38*	−0.23	−0.12	−0.21	0.47*	0.33*	0.26	0.19	0.20

1: Cob weight; 2: Grain weight; 3: Plant height; 4: Stem diameter; 5: Number of internodes; 6: Proportion internodes bored; 7: Number of holes bored; 8: Tunnel length; 9: Cob damage; 10: Stemborer abundance; 11: Parasitism.

host immatures maybe more accessible to parasitoids in the thin-stemmed wild grasses than in large-stemmed cultivated crops. Thus, the role of wild habitats in the naturally occurring control of stemborers attacking crops is widely unknown.

References

Ampofo J.K.O. 1988. Assessment of on-farm losses in maize production due to insect pests. *Insect Science and its Application* 9: 687-690.

Berger A. 1981. *Biological control of the spotted stalkborer, Chilo partellus (Swinhoe) in maize by using the bacteria Bacillus thuringiensis.* Annual report 1979/1980, INIA, Project UNDP/FAO MOZ/75/009, 19 p.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005. Effects of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in the humid forest of southern Cameroon. *Agriculture, Ecosystem and Environment* 108: 17-28.

Cugala D.R., Overholt B.W., Santos L., Giga D. 1999. Performance of *Cotesia sesamiae* and *Cotesia flavipes* (Hymenoptera: Braconidae) as biological control agents against cereal stemborers in Mozambique. *African Crop Science Journal* 7: 497-502.

Cugala D.R., Omwega C.O. 2001. Cereal stemborer distribution and abundance and the introduction and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in Mozambique. *Insect Science and its Application* 21: 281-287.

Cugala D.R., Overholt W.B, Santos L., Giga D. 2001. Trial releases of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) against cereal stemborers in Mozambique. *Insect Science and its Application* 21: 303-310.

Davies G., Cumbi S., Tocoro C. 1995. Brocas de milho. Uma contribuição para o seu estudo no planalto de Lichinga, Niassa. INIA. *Série Investigação* 21: 40.

Ehler L.E., Eveleens K.G., van den Bosch R. 1973. An evaluation of some natural enemies of cabbage looper on cotton in California. *Environmental Entomology* 2: 1009-1015.

Eveleens K.G., van den Bosch R., Ehler L.E. 1973. Secondary outbreak induction of beet armyworm by experimental insecticide application in cotton in California. *Environmental Entomology* 2: 497-503.

Gonçalves M.L. 1970. A broca do milho, *Chilo partellus* (Swinhoe) (Lepidoptera:Crambidae) em Moçambique. Contribuição para o seu estudo. *Agronomia Moçambicana* 4: 239-246.

Gounou S., Schulthess F., Shanower T., Hammond W.H.O., Braima H., Cudjoe A.R., Adklaoe R., Antwi K.K., Olaleye I., 1994. *Stem and ear borers of maize in Ghana.* Plant Health Management Research Monograph 4, International Institute of Tropical Agriculture, Ibadan, Nigeria, 23 p.

Kfir R. 1995. Parasitoids of the African maize stemborer, *Busseola fusca* (Lepidoptera: Noctuidae), in South Africa. *Bulletin of Entomological Research* 85: 369-377.

Kfir R. 1997. Natural control of the cereal stemborers *Busseola fusca* and *Chilo partellus* in South Africa. *Insect Science and its Application* 17: 61-67.

Kfir R. 1998. Maize and grain sorghum: Southern Africa, p.29-37 *in*: Polaszek A. (ed.), *African cereal stemborers. Economic importance, Taxonomy, Natural enemies and Control.* CAB International.

Kfir R. 2000. Seasonal occurrence, parasitoids and pathogens of the African stemborer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), on cereal crops in South Africa. *Insect Science and its Application* 17: 61-68.

Kfir R. 2002. Increase in cereal stemborer populations through partial elimination of natural enemies. *Entomologia Experimentalis et Applicata* 104: 299-306.

Khan N.A. 1993. Activation of 14 C-dimethoate in lepidopterous larvae. *Comparative Biochemistry and Physiology* 105: 63–68.

Kumar H. 1997. Resistance in maize to *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae): Role of stalk damage parameters and biological control. *Crop Protection* 16: 375-381.

Lim G.S. 1970. Some aspects of the conservation of natural enemies of rice stemborer and the feasibility of harmonizing chemical and biological control of these pests in Malaysia. *Mushi* 43: 127-135.

Muturi J.J., Ngi-Song A.J., Mueke J.M., Schulthess F., Jiang N. 2006. Multiparasitism by Pupal Parasitoids, *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae) and *Pediobius furvus* (Hymenoptera: Eulophidae) on African Cereal Stemborer *Chilo partellus* (Lepidoptera: Crambidae) and *Busseola fusca* (Lepidoptera: Noctuidae). *Biocontrol Science and Technology* 16: 49-60.

Ndemah R., Schulthess F., Poehling M., Borgemeister C. 2000. Species composition and seasonal dynamics of lepidopterous stemborers on maize and the elephant grass, *Pennisetum purpureum* (Moench) (Poaceae), at two forest margin sites in Cameroon. *African Entomology* 8: 265-272.

Ndemah R., Schulthess F. 2002. Yield of maize in relation to natural field infestations and damage by lepidopterous borers in the forest and forest/savannah transition zones of Cameroon. *Insect Science and its Application* 22: 183-193.

Ndemah R., Schulthess F., Korie S., Borgemeister C., Poehling M., Cardwell K.F. 2003. Factors affecting infestations of the stalk borer *Busseola fusca* (Fuller) on maize in the forest Zone of Cameroon with special reference to scelionid egg parasitoids. *Environmental Entomology* 32: 61-70.

Overholt W.A., Ogenda A.K., Lammers P.M. 1994. Distribution and sampling of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in maize and sorghum on the Kenya coast. *Bulletin of Entomological Research* 84: 367-378.

Overholt W.A. 1998. Biological control, p. 349-362 *in*: Polaszek A. (ed.), *African cereal stemborers. Economic importance, Taxonomy, Natural enemies and Control.* CAB International.

SAS Institute 1999. *User's Guide: Statistics, software release 6.12.* SAS Institute, Inc., Cary, NC, USA.

Schulthess F., Bosque-Pérez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Goergen, G. 1997. Exchanging natural enemies species of lepidopterous cereal stemborers between African regions. *Insect Science and its Application* 17: 97-108.

Seshu Reddy K.V., Sum K.O.S. 1992. Yield-infestation relationships and determination of economic injury level of the stemborer, *Chilo partellus* (Swinhoe) in three varieties of maize, *Zea mays* L. *Maydica* 37: 371-376.

Segeren P. Rafael E., Sitoi V. 1991. Milho. Principais doenças e pragas. Relatório de Ensaios realizados no regadio do Chokwe, 1986/1990. *Serie Investigação* 10: 15-18.

Sétamou M., Schulthess F. 1995. The influence of egg parasitoids belonging to the *Telenomus busseolae* (Hym.: Scelionidae) species complex on *Sesamia calamistis* (Lepidoptera: Noctuidae) populations in maize fields in southern Benin. *Biocontrol Science and Technology* 5: 69-81.

Schulthess F., Chabi-Olaye A., Goergen G. 2001. Seasonal fluctuations of noctuid stemborer egg parasitism in southern Benin with special reference to *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Telenomus* spp. (Hymenoptera: Scelionidae) on maize. *Biocontrol, Science and Technology* 11: 765-777.

Sinha S. N., Lakhani K. H, Davis B. N. K. 1990. Studies of the toxicity of insecticidal drift to the first instar larvae of the large white butterfly *Pieris brassicae* (Lepidoptera: Pieridae). *Annals of Applied Biology* 116: 27–41.

Van den Berg J., van Rensburg J.B.J., Pringle K.L. 1991. Comparative injuriousness of *Busseola fusca* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Pyralidae) on grain sorghum. *Bulletin of Entomological Research* 82: 137-142.

Release and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) an exotic parasitoid of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in East and Southern Africa

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Abstract. *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was first imported into Kenya in 1991 from Pakistan for control of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). First releases were made at the Kenya coast in 1993 and a few recoveries of the parasitoid were made the following year. Additional foreign exploration for *C. flavipes* was conducted in central India in 1996 and 1998, which resulted in additional importation of the parasitoid for subsequent releases in eastern and southern Africa. Region-wide releases commenced with releases in Mozambique in 1996, Somalia in 1997 and Uganda in 1998. By 2005, many releases had been made in 10 countries in East and Southern Africa with establishment being reported in all of them except Eritrea but including Ethiopia where releases had never been made. This paper describes the progress made in the release and establishment of *C. flavipes* in East and Southern Africa and quantifies the rate of spread from the initial release sites in Kenya.

Résumé. Lâcher et établissement de *Cotesia flavipes* Cameron (Hymenoptera : Braconidae) un parasitoïde exotique de *Chilo partellus* (Swinhoe) (Lepidoptera : Cambidae) à l'Est et au Sud de l'Afrique. *Cotesia flavipes* Cameron (Hymenoptera : Braconidae), originaire du Pakistan, a été importé au Kenya en 1991 pour le contrôle de *Chilo partellus* (Swinhoe) (Lepidoptera : Crambidae). Les premiers lâchers ont été réalisé dans la région côtière du Kenya en 1993 et une faible présence du parasitoïde a été observée l'année suivante dans les zones de lâcher. La recherche d'autres populations de *C. flavipes* a été menée dans les états du centre de l'Inde de 1996 à 1998, ce qui a conduit à l'introduction d'autres populations de ce parasitoïde à l'Est et au Sud de l'Afrique. Les pays concernés ont été le Mozambique en 1996, la Somalie en 1997 et l'Ouganda en 1998. Depuis 2005, plusieurs lâchers ont été réalisés dans dix pays de l'Est et du Sud de l'Afrique ce qui a conduit à l'établissement du parasitoïde dans tous ces pays à l'exception de l'Erythrée, mais incluant l'Ethiopie où pourtant aucun lâcher n'a été effectué. Cet article décrit les progrès réalisés dans le lâcher et l'établissement de *C. flavipes* à l'Est et au Sud de l'Afrique, et quantifie les taux de propagation du parasitoïde depuis son lieu d'introduction au Kenya.

Keywords: Biological control, Stem borers, *Chilo partellus*, *Cotesia flavipes*, Eastern and Southern Africa.

L epidopteran stem borers are a major constraint to maize and sorghum production in East and Southern Africa. The most common species include the noctuids, *Busseola fusca* (Fuller 1901) and *Sesamia calamistis* Hampson 1910 the pyralid, *Eldana saccharina* Walker 1865 and the crambids *Chilo partellus* (Swinhoe 1885) and *Chilo orichacociliellus* (Strand 1911). However, only *C. partellus* and *B. fusca* are key pests and their economic importance varies with altitude (Kfir *et al.* 2002). For example, in Mozambique *C. partellus* is the dominant species at low altitude (below 800 m above sea level [asl]) areas in the south and central parts of the country, while *B. fusca* is abundant at elevations above

800 m asl (Cugala & Omwega 2001). In Zimbabwe, *C. partellus* is dominant at altitudes below 600m asl while *B. fusca* dominates at altitudes above 1200m (Chinwada *et al.* 2001). *C. partellus* is exotic to Africa and is thought to have been introduced from Asia, sometimes before the 1930s (Tams 1932).

Attempts at classical biological control against *C. partellus* using its co-evolved natural enemy *Cotesia flavipes* Cameron 1891 (Hymenoptera: Braconidae) are well documented (Overholt *et al.* 1997). In East Africa, the first attempt was made in the 1970s by the International Institute of Biological Control (IIBC), which, however, did not result in establishment (Overholt *et al.* 1994). A second attempt was initiated by ICIPE in 1991. Releases of *C. flavipes* were made in 1993 in coastal Kenya, where the parasitoid became permanently established and spread to other

areas (Omwega *et al.* 1995). Thereafter, the program was expanded to cover eleven countries in East and Southern Africa. For example, in Mozambique first releases were made in 1996 (Cugala & Omwega 2001), Somalia in 1997, Uganda in 1998 (Matama-Kauma *et al.* 2001) and Zanzibar (Niyibigira 2003) Malawi, Zimbabwe (Chinwada *et al.* 2001), Zambia (Sohati *et al.* 2001) and Tanzania in 1999, and Eritrea in 2003. Except for Eritrea, establishment has been recorded in all the countries where releases were made, including Ethiopia where releases were never made and *C. flavipes* probably invaded from Somalia (Getu *et al.* 2003).

The objective of this paper is to review work conducted through various stages of the biological control project from exploration, rearing, release and establishment of *C. flavipes* in East and Southern Africa and to estimate the rate of spread of the parasitoid in Kenya.
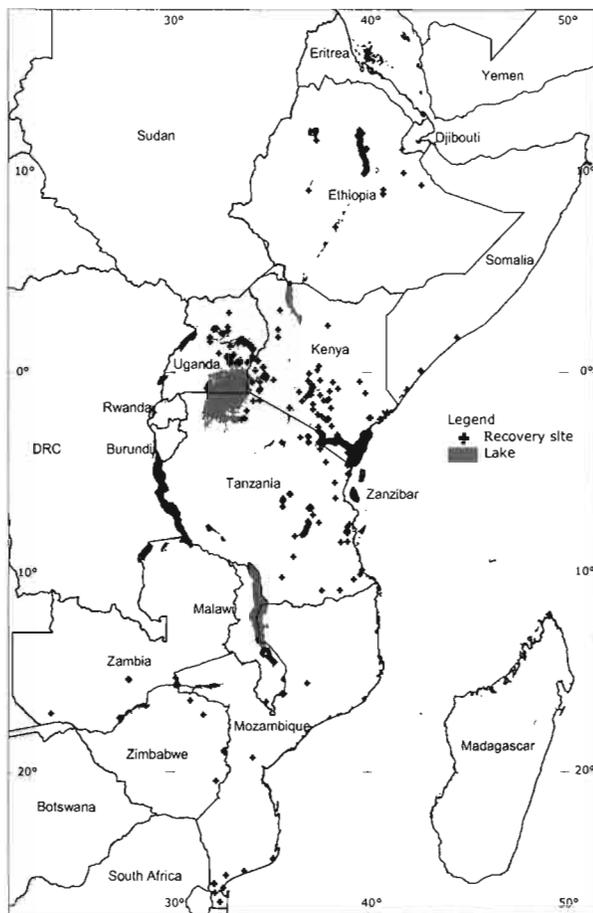
Figure 1
Areas of recovery of *Cotesia flavipes* in East and Southern Africa.

Materials and Methods

Importation of parasitoids from Pakistan

Three populations of *Cotesia flavipes* were collected in Pakistan by the International Institute of Biological Control (IIBC) and shipped to the International Centre of Insect Physiology and Ecology (ICIPE) through the Kenya Agricultural Research Institute (KARI) quarantine station, Muguga, Kenya. The first cocoon masses from Rawalpindi (north Pakistan) arrived in September 1991, the second shipment collected from Sindh (south Pakistan) arrived in June 1992, and the third from Karachi arrived in Kenya in June 1995. The number of the field collected individuals that founded the three populations is not known. The Sindh population was released at the Kenya coast in 1993 (Overholt *et al.* 1994).

Exploration in India

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Asia Centre, Patancheru, was used as the operational centre for all exploration, laboratory rearing and shipment of parasitoids. Extensive searches for parasitoids were carried out between August and September 1996 in India covering the states of Maharashtra, Andra Pradesh and Kanataka . Six districts (Nagpur, Warda, Yavatima, Amravathi, Akola and Buldana) were surveyed in Maharashtra, three districts (Medak, Rangareddy and Mahbubnagar) in Andra Pradesh, and three districts (Bijapur, Gulburga and Dharwad) in the Kanataka state. In all, four exploration trips were made. Two trips to Maharastra state were from August 21 to 27 and August 30 to September 5. One trip in Andra Pradesh lasted from September 9–15 and the trip to Kanataka from September 19–24, 1996. Parasitoid shipments to Kenya from these collections were made in four consignments.

A second exploration in India was conducted between December 11, 1998 and January 2, 1999 in three districts (Warangal, Medak and Mahbubnagar) in Andra Pradesh, two districts (Raichur, Bijapur) in Karnataka and five districts (Solarpur, Osmanabad, Ahmadnagar, Bid and Nanded) in Maharashtra. (Niyibigira 2003). A total of 44 cocoons from this survey were shipped to Kenya and used to found a colony of several isofemale lines and a mixed population which were used for experimental releases in Zanzibar.

The districts were chosen because they had a sorghum crop in the field. Several sorghum fields were searched for signs of stem borer infestation every 10 to 20 km along the main roads. Plants with stem borer damage were excised and dissected at each site. Stem borer larvae removed from the stems were placed in plastic jars and provided pieces of sorghum stems as diet.

The stem borers were checked daily for signs of parasitization or cocoon formation. Larvae that showed signs of parasitization were transferred singly into vials containing artificial diet and reared for parasitoid emergence and identification. Collected insects were brought to the laboratory at ICRISAT, Asia Centre, where progeny emerging from each *Cotesia* cocoon mass was used to initiate a separate colony by allowing sib-mating within the vial and exposing medium sized larvae of *C. partellus* to mated females the following day. Each colony initiated by a single cocoon mass from the field was labeled as a separate isofemale line. The isofemale lines were reared for one generation at ICRISAT before shipment. The freshly formed cocoon(s) from each isofemale line were placed in one vial and labeled according

to the collection site and the vials were packaged and shipped by air to Kenya. On arrival the cocoon masses were taken to the quarantine station at KARI, Muguga, where they were reared for one generation before being released to ICIPE.

Surveys for stem borer species composition and occurrence of indigenous natural enemies of stem borers in East and South Africa

Before parasitoid releases were conducted in any country, country-wide surveys were carried out to determine the stem borer species composition and distribution (Getu *et al.* 2001; Matama-Kauma *et al.* 2001; Niyibigira *et al.* 2001; Nsami *et al.* 2001; Overholt *et al.* 1994). The preliminary surveys were conducted by sampling maize and sorghum fields every 5–20 km along the main roads of a country. At each sampling site a maize or sorghum field with plants at vegetative stage was divided into four quadrats and five plants per quadrat were selected at random and examined. Those that showed signs of putative stem borer infestation were uprooted and dissected to obtain immature stages of stem borers. The medium to large stem borer larvae were sorted by species and placed in plastic jars and provided with pieces of maize or sorghum stems as food. The larvae were checked daily for cocoon formation. Cocoon masses were placed singly in plastic vials and labeled by site and host species. After emergence the parasitoids were sexed and identified. The stem borers were identified at larval stages, wherever possible, or as adults. Several stem borer species were recovered from maize and sorghum fields throughout the region including the exotic stem borer *C. partellus* and several indigenous stem borers including *B. fusca*, *S. calamistis* and *C. orichalcocilliellus*. Areas where *C. partellus* was dominant were targeted for releases of *C. flavipes*.

Field releases of parasitoids

The Sindh population was released at the Kenya coast in 1993 (Overholt *et al.* 1994). After successful establishment in Kenya, the release program was expanded to other countries in East and Southern Africa. The insects for releases throughout the region were mass reared at ICIPE from colonies comprising material collected from Pakistan and India. Parasitoids were reared at ICIPE using a hand-stinging method (Overholt *et al.* 1994). Mated females were allowed to oviposit once in medium-sized *C. partellus* larvae. The stung larvae were then reared in artificial diet (Ochieng *et al.* 1985) until cocoon formation. Freshly formed cocoons were packaged and shipped by air to the country where the releases would be conducted. On arrival, cocoons were transported before or after emergence to pre-selected fields where releases were made. Several release methods were employed depending on exigencies of the field. The most common method was releasing adult wasps. The parasitoids were allowed to emerge then allowed to mate overnight before liberation the following morning. The second method was releasing the parasitoid in cocoon form. Cocoon masses were taken to the field and placed in a release station (Overholt *et al.* 1994) and left to emerge and disperse. The third method was used in Zimbabwe (Chinwada, *pers. com.*) where the mated parasitoids in a cage were taken to the field and allowed to singly sting medium-sized larvae dissected from stems in an infested field and reintroducing stung larvae into the stems of infested plants in the field.

Recovery of parasitoids

After releases were made, field surveys were conducted during the same season to determine whether colonization had taken place. Surveys were continued in subsequent seasons in release and non-release sites to determine whether establishment and spread had taken place. Fields were selected at 10–20 km intervals and a field was divided into four quadrats and five plants selected at random from each and dissected to obtain immature stages of stem borers, which were reared for parasitoid emergence or until pupation.

Spread of parasitoids

To quantify the rate of spread of parasitoids outward from the releases sites, we used survey data for Kenya from 1993 to 2005. Distances traversed by female parasitoids were calculated from release sites to the locations of parasitized larvae and pooled into one data set. A total of 478 recovery locations were identified and their distances calculated from 8 release sites (fig. 1, 2). Dispersal probability (spread tendency) was evaluated by fitting the frequency distribution of parasitoids in relation to the release sites to Taylor's model (Taylor 1978), $N = \exp(a + bX^c)$, where 'N' is the number of individuals dispersing to distance 'X', 'a' specifies the sample size, 'b' is a scale factor which depends upon the units distance is measured in, and 'c' indicates the rate of change in density with distance and is also a measure of non-randomness in dispersal behavior. Then diffusion theory was used to analyze dispersal distance.
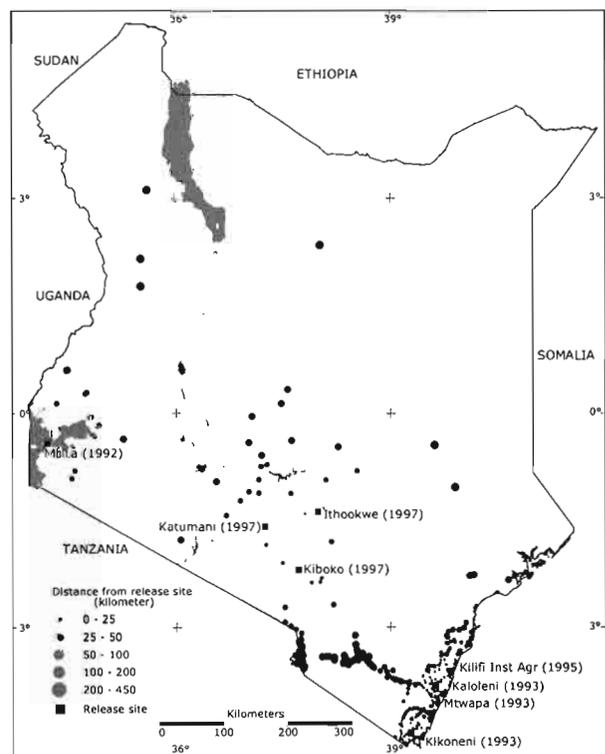
Figure 2
Cotesia flavipes release and recovery sites in Kenya.

Table 1. The number cocoons masses collected from stem borers reared from various sites and districts of Maharashtra and Andra Pradesh states in India.

State	District	Sites sampled	Number cocoons
Maharashtra			
	Maharashtra	5	0
	Warda	4	0
	Yavatima	14	8
	Amravathi	6	13
	Akola	10	10
	Buldana	9	32
Andra Pradesh			
	Medak	6	18
	Rangareddy	8	99
	Mahbubagar	13	41
Total			**221**

Two cocoons masses from Amaravathi and Medak were from *Sesamia inferens*. The rest of the cocoon masses were from *Chilo partellus*.

The speed of population spread has been reliably estimated using diffusion coefficients and intrinsic rates of increase. The appropriate rate for our analysis (Okubo & Kareiva 1980) may be estimated as

$$c = \sqrt{(rD_{an})} \tag{1}$$

where D_{an} is the annualized diffusion coefficient and r is the annual intrinsic rate of increase (Skellam 1951; Okubo & Kareiva 1980).

To calculate the spread of parasitoids, we used estimates of the longevity, $s = 3$ days, (Mbapila & Overholt 2001) and intrinsic rate of increase, $r = 0.1508$, from previous studies (Mbapila & Overholt 2001). From these estimates, the annualized diffusion coefficient was calculated as

$$D_{an} = D * s \tag{2}$$

where $s = 3$ days and is the mean survival time of adult

parasitoids and D is the diffusion coefficient (see below). Finally, the intrinsic rate of increase for parasitoids was used to calculate speed of population spread using equation (1).

We estimated diffusion coefficient (D) from survey data by using the formula:

$$D = \frac{2(MD)^2}{\pi t} \tag{3}$$

where MD is the mean distance traveled and t is the time during which this distance is covered. The mean distance traveled is expressed as

$$MD = \sum_{N-1}^{N} \frac{distance\ of\ recovery\ sites\ from\ release\ sites}{Total\ number\ of\ sites} \tag{4}$$

Results

Exploration in India in 1996, rearing and shipment of parasitoids

Two stem borers, *Chilo partellus* and *Sesamia inferens* Walker 1856 (Lepidoptera: Noctuidae) were collected from all districts of Maharashtra except Nagpur. *Chilo partellus* was collected from the three districts of Andhra Pradesh and *S. inferens* from Rangareddy and Medak but not Mahabubnagar. *C. flavipes* was reared from stem borers collected from the districts of Yavtmal, Amaravati, Akola and Buldana in Maharashtra State. In Andhra Pradesh, *C. flavipes* was found in the three districts surveyed. A total of 221 cocoon masses of *C. flavipes* were reared from the stem borers collected (tab. 1). Isofemale lines were initiated from 183 of the cocoons masses. Broods of the remaining 38 cocoon masses consisted of the same sex. Four shipments were sent to Kenya during the month of September but only three arrived. One shipment was wrongly routed and arrived only after the wasps had emerged and died. After passing through one generation in quarantine,

Table 2. Number and year of Shipment of Cocoon masses of *Cotesia flavipes* to various countries in East and South Africa.

Country	Year and number (X1000) of parasitoids shipped for release												
	93	94	95	96	97	98	99	00	01	02	03	04	05
Eritrea											600	400	150
Kenya	60		250		250						200	800	
Malawi							300	100	75	200	2100	500	300
Mozambique			10			410	410	540	110	100	2300	700	100
Tanzania										300	600	1100	100
Uganda							125	500	100		500	500	
Zambia								225	490		100	900	700
Zanzibar										100	900	500	100
Zimbabwe							50	50	50		100	700	200
Somalia					180								

a total of 88 isofemale lines were established at ICIPE rearing laboratory. This represents of about 47% of the isofemale lines shipped and about 40% of the cocoon masses originally collected from the fields in India.

Release and establishment of *Cotesia flavipes* in Eastern and Southern Africa

The number of cocoons shipped to each country over the years is given in Table 2. The year when releases commenced in each country and the first year of recovery are presented in Table 3. For Tanzania, *C. flavipes* was found in that country in 1995 presumably from releases made in Kenya (Omwega *et al.* 1997). But releases in other areas of the country commenced in 2002.

Estimate of dispersal rate of parasitoids in Kenya

Figure 2 indicates that parasitoids are spreading as a combination of short and long distance dispersal as predicted by Taylor's (1978) non-linear dispersal model. The value of c (−0.0114) indicated a tendency to spread as isolated colonies rather than as an advancing front from release sites. Based on physiological data (Mbapila & Overholt 2001), *C. flavipes* expanded its range by neighborhood diffusion at a rate of 11.23 km per year.

Discussion

Preliminary surveys carried out confirmed the dominance of *Chilo partellus* and *Busseola fusca* among the stem borer infesting maize and sorghum in East and Southern Africa. *C. partellus* dominated at lower altitudes and *B. fusca* at higher elevations. Lower elevation areas were targeted for the release of *Cotesia flavipes*.

The current biological control program is the second attempt at introducing *C. flavipes* as a biological control agent of *C. partellus* in Eastern Africa. The first attempt was by the International Institute of Biological Control (IIBC) who made releases of the parasitoid in Kenya, Uganda and Tanzania between 1968–1972 but the releases did not result in establishment (Overholt *et al.* 1997). Reasons for the recent successful establishment of *C. flavipes* are not clear but may include better targeting of areas where *C. partellus* is abundant. Laboratory studies indicate that releasing the parasitoid where *B. fusca* and/or *Eldana saccharina* are predominant will most likely results in failure to establish since the stem borers are not suitable hosts (Ngi-Song *et al.* 1995; Overholt *et al.* 2003).

Low genetic diversity has been cited as one of the causes of failure of introduced natural enemies to establish (Stouthamer *et al.*1992; Hopper *et al.* 1993). Genetic loss may be occasioned by losses through

various stages of a biological control program. The loss of genetic material was tracked by documenting the fate of isofemale lines initiated from the field collected materials that made it to the mass rearing laboratory at ICIPE after going through shipment and quarantine processing. In the exploration conducted in 1996, a total of 221 cocoon masses were collected in central India and only 40% of this genetic material made the founding colony established at ICIPE.

There is very little field data available on the importance of genetic diversity in colonization of parasitoids. A field study conducted in Zanzibar examined the role of genetic diversity within a population in colonization using three genetically impoverished populations of *C. flavipes* and one population with high genetic diversity (Niyibigira 2003). The results from the study did not show any significant differences between the different populations. In the first releases in Kenya, only the Sindh population from Pakistan was used (Overholt *et al.* 1994) and the establishment at the coast of Kenya indicated release from a single source did not hinder establishment. However, in subsequent releases genetic materials from all the collections from Pakistan and India were used to maximize the genetic diversity of released populations. Based on the different ecologies in the vast areas of release, it was thought that genetically diverse population would increase the potential for successful establishment. The releases that have been conducted over several years have resulted in establishment (Cugala & Omwega 2001; Matama-Kauma *et al.* 2001; Omwega *et al.* 1995) and spread of the parasitoid from the release to the non- release areas (Getu *et al.* 2003; Omwega *et al.* 1997).

In most releases, recoveries were made within the year of release (Overholt *et al.* 1994; Matama-Kauma 2001; Songa *et al.* 2001) but not in subsequent seasons

Table 3. Commencement of releases and recovery year of *Cotesia flavipes* in different countries in Eastern and Southern Africa.

Country	Year of first release	Year of first recovery
Eritrea	2003	-
Ethiopia	None	1999
Kenya	1993	1994
Malawi	1999	2000
Mozambique	1996	1997
Somalia	1997	1999
Tanzania	2002	1995
Uganda	1998	1998
Zambia	1999	2004
Zanzibar	1999	1999
Zimbabwe	1999	2004

until several years later when high levels of parasitism were recorded (Overholt 1998). For example in Kenya the suppressive effects of *C. flavipes* released in 1993 on *C. partellus* was not apparent until 1997–1998 season (Zhou *et al.* 2001). The same phenomena was reported from Zimbabwe where recoveries from 1999 releases were only made in 2004 with high levels of parasitism recorded in some areas (Chinwada et al. *in lit.*). Similarly, in Zambia no recoveries were made from releases of 1999 until 2004 (Sumani *pers. com.*). Reasons for this are not clear but may be due to low numbers which are difficult to detect in the initial phase of establishment (Overholt 1998).

Data on frequency distribution of *C. flavipes* in Kenya between 1993–2003 regressed against distance from release site shows a negative exponential gradient indicating that the parasitoid tended to spread as

isolated populations rather than as an advancing front. This may partly explain why it is difficult to make post-release recoveries until several years after release.

Dispersal out of release sites accelerated over time (fig. 3) so that by the third year parasitoids were expanding out at a rate of about 11 km per year. As the *C. flavipes* life cycle requires approximately 20 days at 25 °C (Ngi-Song *et al.* 1995), this means there are 18 generations of parasitoids per year, and each generation of parasitoids is traveling approximately 34 m. This value agrees well with the observed mean dispersal distance, approximately 30 m, in Sallam's study (Sallam *et al.* 2000), but at least a few female parasitoids are evidently able to travel much further. The increasing rates of dispersal may be due to rare dispersal events becoming more common with increasing numbers of parasitoids or it is possible that rapidly dispersing
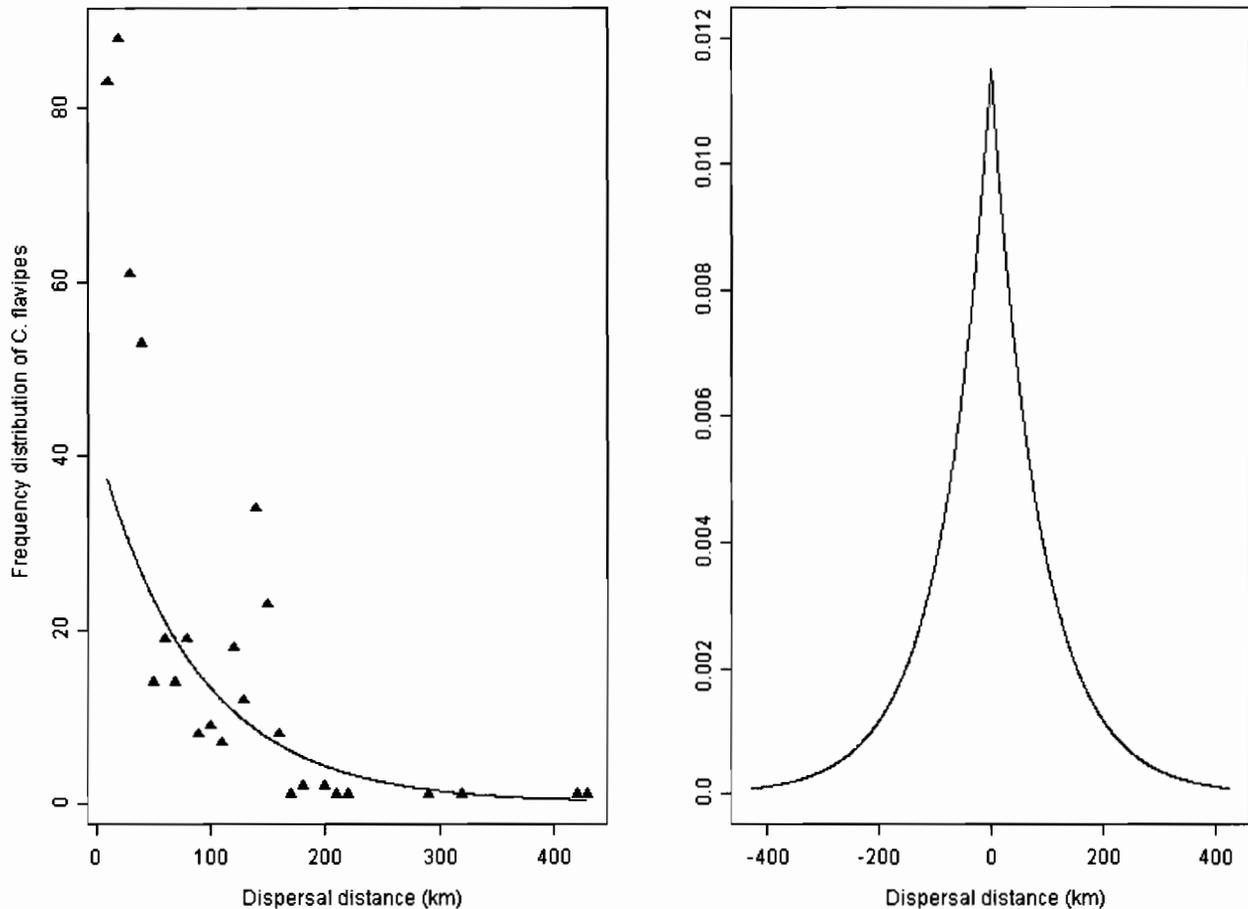
Figure 3
Dispersal curve and redistribution kernel for *Cotesia flavipes*. The curve was taken from Taylor (1978) follows equation: y = 41.77e^-0.0114x, R² = 0.6939. The corresponding redistribution kernel on the right was obtained by mirroring dispersal curve about the origin and dividing by the total area underneath the curve so as to generate a probability density function with total area equal to 1.

parasitoids are being selected for because they are able to colonize new habitat more frequently.

Determining dispersal rates from releases of *C. flavipes* is important because it allows us to predict how many releases would be necessary to cover a given region in a specified amount of time. It also allows us to choose release sites that are spaced at sufficient intervals to fill a region with as few releases as possible.

References

Chinwada P., Omwega C. O., Overholt W.A. 2001. Stem borer research in Zimbabwe and prospects for the establishment of *Cotesia flavipes*. *Insect Science and its Application* 21:327-334.

Cugala D., Omwega C.O. 2001. Cereal stem borer distribution and abundance and the introduction and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in Mozambique. *Insect Science and its Application* 21:281-287.

Getu E., Overholt W.A., Kairu E. 2001. Distribution and species composition of stem borers and their natural enemies in maize and sorghum in Ethiopia. *Insect Science and its Application* 21: 353-359.

Getu E., Overholt W.A., Kairu E., Omwega C.O. 2003. Evidence of the establishment of *Cotesia flavipes* (Hymenoptera: Braconidae), a parasitoid of cereal stem borers, and its host range expansion in Ethiopia. *Bulletin of Entomological Research* 93: 125-129.

Hopper K.R., Rouch R.T., Powell W. 1993. Management of genetics of biological control introductions. *Annual Review of Entomology* 38: 27-51.

Kfir R., Overholt W.A., Khan Z.R., Polaszek A. 2002. Biology and management of Economically important Lepidoptera cereal stem borers in Africa. *Annual Review of Entomology* 47:701-731.

Matama-Kauma T., Kyamanywa S., Ogwang J. A., Omwega C.O., Wilson H.R. 2001. Cereal stem borer species complex and establishment of *Cotesia flavipes* Cameron in eastern Uganda. *Insect Science and its Application* 21:317-325.

Mbapila J.C., Overholt W.A. 2001. Comparative development, longevity and population growth of exotic and native parasitoids of lepidopteran cereal stem borers in Kenya. *Bulletin of Entomological Research* 91: 347-353.

Ngi-Song A.J., Overholt W.A., Ayetertey J.N. 1995. Suitability of African Gramineous stem borers for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Environmental Entomology* 24: 978-984.

Niyibigira E.I., Abdullah Z.S., Lada V.Y. 2001. Distribution and Abundance, in maize and sorghum, of lepidopteran stem borers and associated indigenous parasitoids in Zanzibar. *Insect Science and its Application* 21: 335-346.

Niyibigira E.I. 2003. *Genetic variability in* Cotesia flavipes *and its importance in biological control of Lepidopteran stem borers.* Ph.D. Dissertation, Wageningen University, Wageningen, 162 p.

Nsami E., Pallangyo B., Mgoo V., Omwega C.O. 2001. Distribution and species composition of cereal stem borers in eastern zone of Tanzania. *Insect Science and its Application* 21:347-351.

Ochieng R.S., Onyango F.O., Bungu M.D.O. 1985. Improvement of techniques for mass culture of *Chilo partellus*. *Insect Science and its Application* 6:425-428.

Okubo A., Kareiva P. 2001. Some examples of animal diffusion, p. 170-196 *in*: Okubo A., Levin S. A. (eds), *Diffusion and ecological problems: Mathematical models*. Springer-Verlag, New York, 467p.

Omwega C.O., Kimani S.W., Overholt W.A., Ogol C.K.P.O. 1995. Evidence of the establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in continental Africa. *Bulletin of Entomological Research* 85: 525-530.

Omwega C.O., Overholt W.A., Mbapila J.C., Kimani-Njogu S.W. 1997. Establishment and dispersal of *Cotesia flavipes* (Cameron), an exotic endoparasitoid of *Chilo partellus* Swinhoe in northern Tanzania. *African Entomology* 5: 71-75.

Overholt W.A., Ochieng J.O., Lammers P., Ogedah K. 1994. Rearing and field release methods for *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stem borers. *Insect Science and its Applications* 15: 253-259.

Overholt W.A., Ngi-Song A.J., Omwega C.O., Kimani Njogu S.W., Mbapila J.C., Sallam M.N. , Ofomata V.C. 1997. A review of the introduction and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in East Africa for biological control of cereal stem borers. *Insect Science and its Application* 17: 79–88.

Overholt W.A. 1998. Biological control, p. 349-362 *in*: Polaszek A. (ed.), *Cereal Stem borers in Africa: Taxonomy, Natural Enemies and Control*. CAB International, Wallingford, UK.

Overholt W.A., Conlong D.E., Kfir R., Schulthess F., Setamou M. 2003. Biological control of gramineous lepidopteran stem borers in sub-Saharan Africa, p. 131-144 *in*: Neuenschwander P., Borgemeister C., Langewald J. (eds.), *Biological Control in IPM Systems in Africa*. CABI Wallington, Oxon, UK, 414 p.

Sallam M.N., Overholt W.A., Kairu E. 2000. Dispersal of the exotic parasitoid *Cotesia flavipes* in a new ecosystem. *Entomologia Experimentalis et Applicata* 98: 211–217.

Skellam J.G. 1951. Random dispersal in theoretical populations. *Biometrika* 38: 196-218.

Sohati P.H., Musonda E.M., Mukanga M. 2001. Distribution of cereal stem borers in Zambia and release of *Cotesia flavipes* Cameron, an exotic natural enemy of *Chilo partellus* (Swinhoe). *Insect Science and its Applications* 21:311-316.

Songa J.M., Overholt W.A., Mueke J.M., Okello R.O. 2001. Colonization of *Cotesia flavipes* (Hymenoptera: Braconidae) in stem borers in the semi-arid eastern province of Kenya. *Insect Science and its Applications* 15:253-259.

Stouthamer R., Luck R.F., Werren J.H. 1992. Genetics of sex determination and the improvement of biological control using parasitoids. *Environmental Entomology* 21: 427-435.

Tams W.H.T 1932. New species of African Heterocera. *Entomologist* 65: 1241-1249.

Taylor R.A.J. 1978. The relationship between density and distance of dispersing insects. *Ecological Entomology* 3: 63-70.

Zhou G., Baumgartner J., Overholt W.A. 2001. Impact assessment of an exotic parasitoid on stem borer (Lepidoptera) population dynamics in Kenya. *Ecological Applications*. 11: 1554-1562.

Biological control of cereal stem borers in Kenya: A cost benefit approach

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Abstract. Lepidopteran stem borers are the key pests of maize in Sub-Saharan Africa. In the lowland tropics, dry mid-altitude, dry transitional and the moist mid-altitude zones of Kenya, the invasive crambid *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) causes up to 73% yield loss. The International Centre of Insect Physiology and Ecology (ICIPE) started a biological control (BC) program in 1991 to control stem borers in subsistence agriculture in Africa with emphasis on classical BC of *C. partellus*. The project released the braconid larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in 1993 in coastal Kenya, where it got established and spread to other regions. This study assesses the economic impact of the introduced parasitoid. Temporal data on percentage parasitism by the introduced parasitoid and on stem borer density were collected between 1995 and 2004. Socio-economic data was collected through administration of questionnaires to 300 farmers. Economic impact of the project was calculated as the value of the yield loss abated by the parasitoid based on a model of expected stem borer density and parasitism level. Average annual parasitism increased linearly from the time of introduction to reach 20% parasitism by 2004. The net reduction in total stem borer density over the last 10 years was 33.7%, thus abating 47.3% of yield loss. The region will accumulate a net present value of US $ 183 million in economic benefits in 20 years since release of the parasitoid. Introduction of other parasitoid species targeting the egg and pupal stages of the stem borer life cycle stages would be required for biological control to push yield loss by stem borers to an insignificant level.

Résumé. Contrôle biologique des foreurs des céréales au Kenya : une approche économique. Les lépidoptères foreurs de graminées sont des ravageurs importants du maïs en Afrique subsaharienne. Dans les zones de faibles et moyennes altitudes du Kenya, le foreur exotique *Chilo partellus* (Swinhoe) (Lepidoptera : Crambidae) a causé jusqu'à 73% de perte de rendement. Le Centre international pour l'étude de la physiologie et de l'écologie des insectes (ICIPE, son abréviation anglo-saxonne) a initié en 1991 un programme de lutte biologique classique pour lutter contre ce ravageur. Ce programme a permis la libération en 1993 d'un parasitoïde braconide, *Cotesia flavipes* Cameron (Hymenoptera : Braconidae), dans la région côtière du Kenya, où il s'est établi et à partir de laquelle il a colonisé d'autres régions. Cette étude détermine l'impact économique de l'introduction de ce parasitoïde. Des données temporelles sur le pourcentage de parasitisme et sur la densité de foreurs ont été collectées entre 1995 et 2004. Les données socio-économiques ont été obtenues à l'aide d'un questionnaire diffusé auprès de 300 fermiers. L'impact économique a été calculé à partir des données de perte de rendement inférées par un modèle d'estimation de la densité de foreurs et du taux de parasitisme. Le niveau moyen de parasitisme a augmenté linéairement au cours du temps depuis l'introduction du parasitoïde pour atteindre 20% de parasitisme en 2004. La réduction nette en densité totale de foreurs a été de 33,7% lors de cette dernière décennie, réduisant de 47,3% la perte de rendement. La région devrait accumuler un bénéfice net économique de 183 millions de dollars américains dans ces 20 dernières années depuis que le parasitoïde a été lâché. L'introduction d'autres espèces de parasitoïdes actives contre les oeufs et chrysalides des foreurs devrait permettre de renforcer le contrôle biologique afin de rendre les pertes de rendement causées par les foreurs insignifiantes.

Keywords: Stem borer, biological control, parasitoids, benefits, costs.

Economic growth theory holds that technology change is the primary driver of long-term economic growth and improvement of human welfare. However, the extent to which a technology will improve the welfare

depends on the adoption of the technology, which depends among other factors on the economic benefit derived from adoption. In spite of the significance attached to maize production by farmers in the low potential maize production areas of Kenya, as seen in the quantity of farm resources, especially land, allocated to it (Owuor 2002), adoption of improved varieties, inorganic fertilizers and pesticides in maize fields is low

(Hassan 1998). Factors such as lack of information, finance and labour, which vary amongst farmers and farm characteristics, have been used to explain the slow, partial or absent adoption of technologies. The low adoption of improved technologies and low and unreliable rainfall partially explains why farmers in the area obtain low yields. The low potential areas account for only 11% of the 2.3 millions tons of maize produced nationally although it comprises 29% of the total area under maize in Kenya (De Groote 2003a). In these areas, lepidopteran stem borers cause losses of up to 73% (Seshu Reddy & Walker 1990; Overholt *et al.* 1997; De Groote *et al.* 2003a).

Five stem borers species, the noctuids *Busseola fusca* (Fuller 1901) and *Sesamia calamistis* Hampson 1910, the crambids *Chilo partellus* (Swinhoe 1885) and *Chilo orichalcociliella* (Strand 1911), and the pyralid *Eldana saccharina* Walker 1865 attack maize in Kenya. In the low potential areas, *C. partellus*, an exotic species that invaded eastern Africa from Asia before the 1930s (Tams 1932), is the predominant stem borer accounting for 80% of the species (Overholt *et al.* 1997). The first attempt to control cereal stem borers using the classical BC approach involved the importation of nine species of parasitoids of *C. partellus* from Asia by the Commonwealth Institute of Biological Control (CIBC). The parasitoids were released in Uganda, Kenya and Tanzania from 1968–1972 (CIBC 1968–1972). Later, 13 exotic parasitoids targeting cereal stem borers were released in South Africa in 1977 (Kfir 1994). None of these projects reported establishment

of the parasitoids. A new BC program against *C. partellus* was launched by the International Centre for Insect Physiology and Ecology (ICIPE) in 1991, with funding from the Dutch government. The project imported the braconid *Cotesia flavipes* Cameron 1891, an endo-parasitoid of larvae of cereal stem borers, from Asia in 1991. After assessing the range of locally occurring host species, the parasitoid was released in coastal Kenya in 1993 where it got fully established and from where it spread to other regions including Tanzania (Omwega *et al.* 1995; Omwega *et al.* 1997; Overholt *et al.* 1997). Since then, the parasitoid was released and became permanently established in nine countries in East and Southern Africa (Omwega *et al.* 2006). The biological control (BC) has a high potential to cause economic development through improvement of maize yields across all farm households, since the BC agent spreads to all farms indiscriminately and, thus, no investment is expected from the farmers once the parasitoid is established.

The objective of the present study is to assess the economic impact of the establishment of *C. flavipes* in Kenya. This study is the first attempt to measure economic benefits of the introduction of the parasitoid, twelve years after first establishment in Kenya. Economic impact of the BC project is measured as the value of the yield loss abated by comparing the actual yield loss with what the yield loss would have been if the parasitoid had not been released. An economic evaluation will provide justification for making decisions about future investments in BC programmes.

Materials and methods

Conceptual framework

Impact assessments strive to answer the question, what would have been without the intervention. This can be done by comparing the situations before and after an intervention has been introduced or with and without the intervention (Baker 2000). Production theory states that a change in the use of farm inputs will cause a change in output along the same production function given that no technological change occurs. Technological change accounts for growth in output that is not accounted for by growth in physical input use, reflected as a shift of the production function. The diagrammatic representation in figure 1 shows the theoretical maize production system in Kenya. Y is the maize output while X represents a basket of inputs used to produce maize. TPP is the total physical product. The curve TPP_1 shows the maximum total physical product of an average farm in absence of the stem borer problem, given an input level X, using the available technology and the prevailing weather conditions. However, farmers are only able to achieve Y_3 maize in any production year and operate at the production function TPP_3. The difference in yield $(Y_1 - Y_3)$ denoted by w is lost to stem borers and will vary with stem borer density during any production season. Unless farming technologies change, farmers will always operate on TPP_3 production function
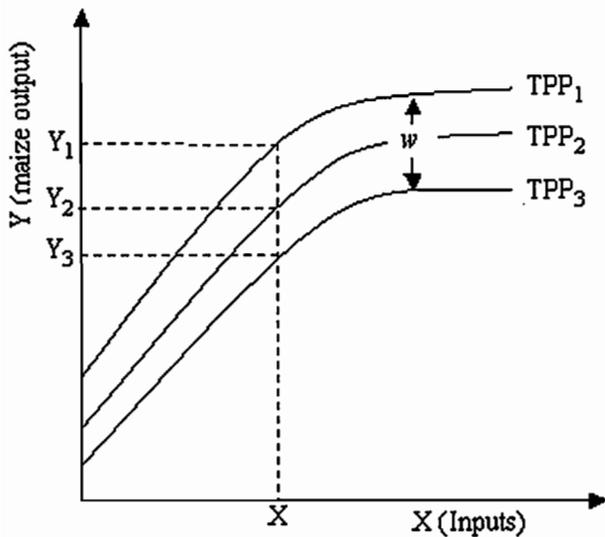
Figure 1
Impact of biological control on maize production.

producing varying quantities of maize depending on the level of inputs used. The introduction of the parasitoid *C. flavipes* would reduce a proportion of the pest and thereby of the yield loss caused by it. This is a change in technology that causes a shift in the production function to TPP$_2$.

The reduction of pest densities as a result of introduction of the BC agent and maize yield loss abated can be assessed directly through yield loss assessments in experimental fields (FAO 1995) and via farmers' interviews (Macharia *et al.* 2005). In this study, it was not possible to categorize farmers into users and non-users of BC in order to determine the impact of the parasitoid on maize yield amongst the farmers because the BC agent spread to all maize fields. Farmers also could not recall pest densities and maize yields in the past 13 years and the baseline data in terms of yield loss attributed to stem borers at the start of the project and corresponding temporal changes were not available. We therefore had to determine how the pest situation would have been without the parasitoid using pest and parasitoid models based on long-term data from the release areas and compare the associated density dependant yield losses with actual yield losses. Yield loss abated was assessed using established pest density-yield loss functions. Determining benefits (yield loss abated) of the project gives a general indication of the attractiveness of the technology to the farmers while evaluation of benefits and costs incurred by the project will help to establish the returns to investment by ICIPE and the funding agency.

In order to carry out the economic analysis based on this framework, several assumptions were made. It was assumed that technological changes do not occur during the period under analysis. The high poverty level was assumed to continue limiting farmers' ability to adopt purchased inputs. This is because resource constraints play an important role in explaining non-participation in markets for inputs by farmers (Omamo 1998). Because the ability of poor farmers to invest in soil nutrient amendments is limited (Freeman & Coe 2002), the low rate of fertilizer inputs use in the region (Wekesa *et al.* 2003) was assumed to maintain and not to improve fertility levels during the period after introduction of the biological control. Other factors outside this model such as improvement of farming skills arising from increasing farming experience were assumed to improve yield by 10 % every 10 years. Under these assumptions, it was possible to compute the change in maize yield resulting from change in stem borer density. Increases in the investment in the agricultural sector that influence the efficacy of farmer education, access to credit, inputs and markets may affect the validity of these assumptions. However, with the continued reduction in investment in agriculture by the government, who is a regulator of the agricultural sector in Kenya, the assumptions are expected to hold over the study period.

Yield loss assessment

Since the early 1970s, the stem borer density in the study area increased up to 1998 (Zhou *et al.* 2001). During this time, *Chilo partellus* was found to be displacing indigenous stem borers to become the most important species (Zhou *et al.* 2001). Seshu Reddy & Walker (1990) reported yield losses due to *C. partellus* ranging from 4-73%. The magnitude of the damage is influenced by soil fertility levels (Sétamou *et al.* 1995; Chabi-Olaye *et al.* 2005a), farming systems (Schulthess *et al.* 2004; Borgemeister *et al.* 2005; Chabi-Olaye *et al.* 2005b) and maize

cultivars (Seshu Reddy & Sum 1991; Ajala *et al.* 2003). In this study, factors, which influence yield loss caused by stem borers, were assumed to be constant.

Several studies have shown that grain weight loss caused by stem borers is linearly related to borer numbers (Usua 1968; Bosque-Pérez & Marek 1991; Seshu Reddy & Sum 1991; Gounou *et al.* 1994; Ajala & Saxena 1994; Sétamou *et al.* 1995; Ndemah & Schulthess 2002). Given that soil fertility remains constant, the biological control program will therefore lead to a linear proportion of yield loss abatement, thus, farmers will move to a higher production function TPP$_2$ from their original production function TPP$_3$. The magnitude of the shift in the production function will depend on the percentage pest control achieved by the parasitoid.

Zhou *et al.* (2001) found linear models of first order autoregression form to be adequate for description of the reduction in stem borer density resulting from parasitism by *Cotesia flavipes*. Other model specifications that could be used to predict the stem borer densities and parasitism were the modifications of Nicholson-Bailey and the Lotka-Volterra models (Pielou 1977). Estimating parameters of these models would have been difficult because of the limitation of our data and complexity of the models. The field data used in this study had initial parasitism of zero before the introduction of *C. flavipes*, a condition that could not be accommodated by these models. The parameters of our model are easy to compute and the model meets our objective of capturing the impact of the introduced parasitoid on pest densities. Following Zhou *et al.* (2001) it was hypothesized that the density of total stem borers at time t depended on the density during the previous period *(t-1)*, parasitism during the current period (t) and the impact of time on stem borer-parasitoid association captured by T starting with the long rains of 1995 ($T = 1$), when the parasitoid showed to have a significant impact on stem borer density. For the parasitoid model, the regressants were the number of seasons elapsed since introduction of *C. flavipes* (T), stem borer density (D_{t-1}) and parasitism during the previous season (P_{t-1}). The general host-parasitoid interaction models used to predict density of stem borers $(D_t) = a + aD_{(t-1)} + bP_t + cT$ while parasitism by *C. flavipes* $(P_t) = \beta + dP_{(t-1)} + eD_{(t-1)} + fT$, where D_t is the mean density of the stem borer complex during season *t*; P_t is the percentage parasitism of stem borers by *C. flavipes* at *t*; *a*, *b* and *c*, are slopes of the stem borer model while *d*, *e* and *f* are the slopes of the parasitism model estimated using the step-wise regression procedure; α and β are the intercept of stem borer and parasitism models; parameter *c* and *f* represented the time dependence of stem borer population dynamics and parasitoid impact, while *a*, *d* and *e* represented the time-delayed stem borer and parasitoid impacts. Stem borer density without parasitism (D_{nt}) was obtained by setting parasitism at zero in the pest model.

The linear models were used to predict P_t, D_t and D_{nt} within the data range of 10 years starting in 1995 and results extrapolated to 20 years. The density and parasitism levels for each year were the projected average for the short (April-June) and long (October-December) seasons of that year. The yield loss abated attributed to stem borer control was computed based on the borer density reduction attributed to parasitism. A larval density of 2 per plant lead to a grain yield loss of about 35% while 6 stem borers cause about 90% loss (Usua 1968; Mailu 1997). The level of yield loss was obtained by constructing a curve for the percentage yield loss against stem borer densities.

With predicted D_t and D_{nt}, the actual and expected output loss were obtained from the curve as $f(D_t)$ and $f(D_{nt})$ respectively. The maize output loss abated attributed to the parasitoid was therefore $f(D_{nt}) - f(D_t)$ denoted by w.

Other benefits of the project

Apart from the yield loss abated, there are several other benefits of the project. Yield loss abatement improved food security and led to increase in caloric intake by households as a results of increased maize output in the regions and the reduction in food poisoning resulting from reduced aflatoxins contamination of ears damaged by stem borers (Sétamou *et al.* 1998; Turner *et al.* 2005). Increase in farm income was also expected to result in possible resource reallocation to farming enterprises to reflect the change in relative profitability of maize in farming households. However, at this stage, the information required to price these benefits is missing, thus, in this study, the benefits are confined to yield losses abated.

Project Costs

Total project costs as incurred by ICIPE, were evaluated to determine the actual costs of the biological control programme. Project costs include cost of scientists, administrative and technical costs, baseline research, foreign exploration, shipping, quarantine processing, mass rearing, field releases, post release evaluation and the cost of acquiring equipment and vehicles necessary for project activities. The costs of supporting graduate training programs were excluded.

Study area, sampling and data collection

Kenya is divided into six agro-ecological zones: the low-land tropics, dry mid-altitude, moist mid-altitude, dry transitional, moist transitional and the highland tropics (Hassan 1998). The agricultural potential of the land increases in that order from the low-land tropics to the high tropics. The introduced parasitoid has spread to 4 agro-ecological zones that fall between the low-land tropics and the dry transitional zones (Zhou & Overholt 2001). All four zones were included in this study. These zones experience a bi-modal rainfall pattern, with the long rains from April to June and the short rains from October to December. The area includes 26 administrative districts of Kenya covering 29% of total area under maize production (De Groote *et al.* 2003a). Farmers in these areas are essentially subsistence maize producers.

This research used both primary and secondary data. Primary data was obtained through administration of a questionnaire to randomly selected farmers after the harvest of 2004 long rains. Five districts were selected randomly from the list of districts, where the introduced parasitoid had spread to as: Kwale and Kilifi in coastal Kenya, Machakos and Makueni in Eastern Kenya, and Siaya in western Kenya. In each of the districts, 2 locations and then two sub-locations per location were randomly selected. The sub-locations chosen were Mpongwe and Perani in Kwale, Chonyi and Bamba in Kilifi, Masii and Tawa in Machakos, Yeekanka and Kimundu in Makueni, and Sega and Boro in Siaya. A list of farmers in each sub-location was then compiled and 15 farmers were selected randomly from the list to give a sample size of 300 farmers.

The primary data collected were input prices, maize yields (90 kg bags), maize price (per 2 kg tin), stem borer infestation scored on a scale of 1–5 (highest infestation 1 and lowest 5), maize quality indicator (i.e. grain rot on a scale of 1–5; highest rotting 5, low rotting 1), causes of low yields, farmers' awareness of the biological control program and their view of the impact of the parasitoid. Grain rot levels were estimated as the proportion of discoloured maize. Stem borer density and parasitization rates over time were obtained from ICIPE's biological control project's data bank at the headquarters in Nairobi. District maize production levels and the area allocated to maize production were obtained from districts' annual reports.

Results and Discussion

Socio-economic characteristics of the farming system

Maize prices in the region exhibited a cycle with the lowest price of Kenyan shillings (Kshs) 20 per 2 kg tin (Kshs 900/90 kg bag) during harvest and an increase to Kshs 40 per 2 kg tin (Kshs 1800/90 kg bag) as maize stocks declined just before the following harvest. The yields obtained by farmers were variable with 80% of the farmers producing an average of 0.6–1.1 ton/ha. All the respondents acknowledged the yield loss as a result of infestation by stem borers. When farmers were asked to rank the major causes of yield loss on a scale of 1–5, stem borers were ranked first and second by 29 and 31% of the respondents, respectively. In lowland tropics of coastal Kenya, drought stress during maize growing seasons and the impact of stem borers were made responsible for crop failure in 1 out of 4 successive seasons and, therefore, the probability of no harvest or low yields during any cropping season was

Table 1. Crop production environment and use of farm input.

Variable	Description	Percentage of farmers
Yields obtained	0.6–1.1 tons/ha	76.0
	< 0.6 tons/ha	20.0
Fertilizer used at planting	Inorganic fertilizers	36.2
	Organic fertilizers	63.8
Pest control method	Pesticides	13.7
	Soil	52.3
	Ash	20.4
	No control	13.4
Farming system	Maize mono-crop	9.4
	Maize +1 intercrop	12.8
	Maize +2 intercrops	23.4
	Maize +3 intercrops	38.6
	Maize +4 intercrops	15.8
Major cause of yield loss ranked 1ˢᵗ by farmers	Inadequate and unreliable rainfall	50.0
	Stem borers	29.0
	Low input use	9.8
	Poor seed quality	11.2

0.25. Over 90% of the farmers practiced intercropping as insurance to food security. There was generally low usage of pesticide to control crop pests with only 13.7% of farmers using commercial pesticides to control pests on maize; application of soil was the most frequently used method of control of stem borers. Soil or ash was impetuously applied often during weeding only to plants that showed obvious symptoms of stem borer attack and therefore, labour input for the application did not increase the cost of maize production. Farmers who did not control the stem borers obtained yields that were not significantly different from that obtained by farmers using soil (t = –0.51, P = 0.63) and ash (t = 1.01, P = 0.32) to control the pest. Thus, the 'no control' group is considered as appropriate baseline for comparison. With exception of the farmers involved in the project none was aware of the introduced parasitoid.

Stem borers damage to the husk allows water to enter into the cob creating a conducive environment for fungal growth. Thirty percent of the respondents reported that maize rotting had reduced at the time of data collection. Ear rot was rated at 3 in the 1990s but reduced to 2 after 2000 on a scale of 1 to 5. There was a significant (P < 0.05) negative correlation between stem borer density and the number of man-days spent on weeding (–0.29, P < 0.05) and the number of intercropped food crops, especially non-host plants of stem borers (–0.41). *Chilo partellus* larvae migrate to the whorl from where they disperse to other plants. It is suggested that in weed free fields migration related mortality was higher than in weedy fields, especially if some of the weeds are alternative hosts, e.g. grasses, to the borer (Ofomata *et al.* 2000; Sétamou *et al.* 2005; Jiang & Schulthess 2005). The negative effect of intercropped and pest infestation has also been described by Schulthess *et al.* (2004) and Chabi-Olaye *et al.* (2005b) and was attributed to the reduced host finding capacity by the ovipositing female moth because of mix-up of plant volatiles (Gohole *et al.* 2003). Correlating the stem borer infestation with yields estimated by farmers gave a significantly negative correlation (–0.61; P < 0.01) corroborating results from yield loss trials carried out in the area (De Groote *et al.* 2001).

Predicting stem borer density and parasitism

Durbin h statistic was first computed to determine whether variables in the parasitioid and host models exhibited serial correlation. The computed Durbin h statistic ranged between 0.04 and 0.11 for the two models. Since the computed values were lower than the critical value of 1.645 of the normal distribution

at the 5% level, there was no reason to reject the null hypothesis of no serial correlation.

The step-wise regression showed that the number of seasons elapsed since introduction of the parasitoid, total stem borer density and parasitism by *Cotesia flavipes* during the preceding season significantly (P < 0.1) affected stem borer density. Parasitism was related positively to time since introduction of *C. flavipes* and parasitism during the previous season. This was due to a reduction of pest density and indicates a negative relationship between parasitism and pest density. Negative density dependence is common for efficient parasitoids and is also found for *Telenomus* egg parasitoids on *S. calamistis* and *B. fusca* (Sétamou & Schulthess 1995, Chabi-Olaye et al. 2005c).

Predicted mean parasitism ranged between 1.2% and 27.5% reducing stem borer densities by between 5.3 and 29.3% from the time of introduction to 2004. Results in figure 2 show that although the introduced parasitoid was firmly established by 1995, total stem borer density continued to rise up to 1998.

The increase in *C. partellus* density up to 1998 (Zhou *et al.* 2001) requires some discussion. Although *C. partellus* was introduced to coastal Kenya in 1930s, it was only reported in 1960s (La Croix 1967). Mean densities in the late 60s to early 70s were around 0.5 (Mathez 1972). Since then it spread and is steadily increased until 1998 (Zhou *et al.* 2001), and Jiang *et al.* (2006) suggests that the pest-parasitoid system is not yet at equilibrium. In addition, as suggested by Schulthess *et al.* (1997) and Zhou *et al.* (2001), the increase in the stem borer density could have been a response to the increase in acreage of maize, which as a food source is considerably superior to wild host plants (Shanower *et al.* 1995; Jiang & Schulthess 2005). Available statistics do not give any evidence for an

Table 2. Stepwise regression results of factors affecting stem borer density and parasitism by *Cotesia flavipes*.

Variables	Dependent variable	
	Parasitism by *Cotesia flavipes*	Stem borer density
Constant	–0.003 ± 0.01	1.07 ±0.15*
Number of seasons elapsed since release	0.15 ± 0.01*	0.31 ± 0.10*
Total stem borers at t-1	–0.01 ± 0.006*	0.20 ± 0.09**
Parasitism by *C. flavipes*	-	–2.67 ± 1.63*
C. flavipes parasitism at t-1	0.63 ± 0.012*	-
F	16.9	16.2
R^2	60.5	42.8

** Significant at p < 0.05 * Significant at p < 0.1; - variable not included in the model.
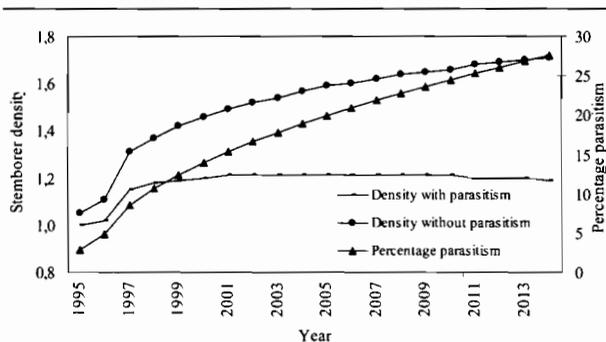
Figure 2
Impact of parasitism on the stem borer density.

increase in area under maize in the 1990s. However, Hassan (1998) found a highly positive correlation between farmers planting maize in both long and short rains and human population density. Thus it can be expected that in response to the high rate of population growth of 2.56% in Kenya (CIA 2005), households intensify production by increasing the

number intercropped crops and farming times per year to meet food demand that would have lead to higher maize production in the area.

Benefits and costs of the project

The percentage reduction in stem borer density arising from parasitism by the introduced parasitoid increased from 5.3% in 1995 to 29.0% in 2004. The model predicts that without release of the parasitoid stem borer density was expected to increase and mean yield losses were expected to reach 34.0% by 2014 (tab. 3). However, predicted yield loss will only be 14% due to a reduction of borer densities caused by the action of the parasitoid. The economic benefits are expected to continue flowing as long as the farming environments, which affect the host-parasitoid system, remains unchanged.

The present value of the cost incurred in Kenya by the project up to 2005 for the BC program was estimated at US $ 4.4 million. Most of the costs (51%) were incurred between 1991 and 1997. During this time, the project was acquiring necessary equipments

Table 3. Predicted impact of *C. flavipes* parasitoid on maize production.

Year	% reduction in stem borer density	Actual output*	Potential output**	Expected % output loss[λ]	Realized % output loss[δ]	Present Value of cost ('000 US$)	Net Present Value (Million US$)
1991						303.8	-0.3
1992						293.4	-0.6
1993						1472.6	-2.1
1994						1082.1	-3.2
1995	5.3	321161	346731	9.3	7.4	1006.1	-3.3
1996	8.8	324372	353531	11.6	8.2	1043.0	-2.9
1997	13.8	324726	374553	19.3	13.3	914.1	-1.0
1998	16.8	328291	382381	21.5	14.1	878.4	1.7
1999	19.3	331855	388867	23.3	14.7	815.9	5.1
2000	21.7	335420	394625	24.7	15.0	741.7	9.2
2001	23.8	339017	399942	25.9	15.2	172.7	14.4
2002	25.6	342582	404870	27.0	15.4	221.8	20.1
2003	27.5	346147	409527	27.9	15.5	207.5	26.3
2004	29.0	349712	413966	28.7	15.5	189.7	33.0
2005	30.6	353277	418224	29.5	15.5	174.5	40.1
2006	32.2	356210	421586	30.2	15.5	158.6	48.5
2007	33.7	359605	425366	30.8	15.5	144.2	58.3
2008	35.2	362999	429036	31.4	15.4	131.1	69.6
2009	36.6	366394	432612	31.9	15.3	119.2	82.7
2010	38.0	369788	436105	32.4	15.2	108.3	97.8
2011	39.4	373183	439524	32.9	15.1	98.5	115.1
2012	40.8	376578	442878	33.3	15.0	89.5	134.9
2013	42.1	379972	446172	33.7	14.8	81.4	157.5
2014	43.5	383367	449413	34.1	14.7	74.0	183.4

*Actual output (tons) obtained by farmers extrapolated after 2005 (Republic of Kenya 1995-2004) **Potential output (tons) assuming complete stem borer control, i.e. 100% yield loss abatement [λ] Yield loss expected when assuming no parasitism [δ] Yield loss after establishment of *C. flavipes*.

for insect rearing, parasitoid release, lab studies and monitoring. Fixed equipments comprised 7–45% of the annual costs of the project. The project cost decreased after 2001 (tab. 3) after the successful establishment of the parasitoid and the project activities were reduced to monitoring and evaluation. By the end of the 20-year period, the biological control program will have accumulated a total net present value (NPV) of US $ 183.4 million using the 10% interest rate. The internal rate of return (IRR) of the project is 41% with the benefit-cost ratio of 19:1 when 2004 farm gate prices are used.

The benefit-cost ratio of the project is lower than that obtained by other BC programs in Africa, e.g., the coffee mealybug with a ratio of 202:1 (Huffaker *et al.* 1976), the cassava mealybug with a ratio of 149:1 (Norgaard 1988), the mango mealybug in Benin with a ratio of 145:1 (Bokonon-Ganta *et al.* 2002), water hyacinth with a ratio of 124:1 (De Groote *et al.* 2003b) and the cabbage diamondback moth in Kenya with a ratio of 24:1 (Macharia *et al.* 2005). However, a large proportion of the classical BC successes against insects were against mealybugs, whose parasitoids are highly specific, and their impact is, thus, much faster than shown in the present project. Furthermore, the low benefit-cost ratio resulted from the limited production quantities resulting from the relatively small project area and the low maize prices. Since the farmers in the project area are subsistence producers who rarely import maize from other regions, farm gate prices (US $ 125.7/ton) were used. This price is lower than the cost insurance and freight (CIF) maize price of about US $ 267/ton used by most studies. Unlike other BC programs in Africa, this study covered only about 400,000 ha of maize production area in Kenya. The project area is small compared to the area covered by other projects; for example the cassava mealybug project whose benefits were extrapolated to the whole of Africa. The benefit-cost ratio will increase when

a complete impact assessment covering all the areas, where the introduced parasitoid has spread to, are included.

The project benefits were also only confined to yield loss abated while there could be other project benefits to the environment and farmer health resulting from reduction of the externalities of pesticide use and increase in household food intake resulting from increase in maize output, whose values are yet to be established. It is worth noting also that the project costs and benefits were for operations in Kenya though 11 countries in East and Southern Africa have benefited from the project through a deliberate release of the parasitoid into these countries or through cross border spread.

Sensitivity analysis

A sensitivity analysis was conducted to test the impact of the variation of the factors that were held constant during the analysis and may affect the results of the economic impact assessment. Over the twenty-year analysis period, it is possible that technological changes might occur. Farmers' resource level may not change much but new advances in technologies, e.g. high yielding varieties, inorganic fertilizers and biotechnology will provide avenues to increase farm output with the same farm resources. Technological change will require farmers to invest money in order to use the technology. For African cereal stem borers, it was shown that in spite of increase in pest density, the net impact of N application on yields is always positive and yield losses due to the pest decreased with increasing nitrogen dosage (Sétamou *et al.* 1995; Chabi-Olaye *et al.* 2005a). Horst & Härdter (1994) found that the net increase in maize yields was 31–42% with 80 kg N ha⁻¹ and 17–34% increase in maize grain when N was supplied to maize field. In relation to this study, the net economic impact of N application will be the decrease in both the potential and the actual yield loss to stem

Table 4. Sensitivity analysis of results of the economic impact of biological control of stem borers.

Parameter	Baseline	Alternative	NPV[1] (Million US $)	BCR[2]	IRR[3]
Interest rate	10%	20%	173.2	9	23
	10%	5%	214.6	20	56
Area under maize	Constant	Increase by 20%	212.2	21	42
Nitrogen fertilizer	No	20% adoption	62.2	6	39
Maize output price	Variable	Increase by 10%	197.8	20	78
Yield loss abated	Depends on pests density controlled	Increase by 10%	197.8	20	78
Period of analysis	20 years	30 years	496.2	46	42

Results of baseline variable: NPV Million US $ 183.4, BCR 19, IRR 41%. [1]Net Present Value, [2] Benefit-cost ratio, [3] Internal rate of return.

borers, which will decrease the economic benefits of the biological control program. If we take an optimistic adoption rate of 20% for purchased inputs, application of N fertilizers that increases yields by 20%, the NPV of the US $ 183.4 million will decrease by 3.2% to US $ 177.6 million.

Application of pesticides may improve maize yields by reducing the yield loss due to stem borers. To assess the impact of change in adoption levels of pesticides to the results of out study requires data on both target and non-target impact of pesticides. Data is required to estimate the benefits of a pesticide compared to its costs cover environmental risks of pesticide use such as persistence in soil and water, contamination groundwater, residues in and on food and hazards to non-target organisms and costs incurred by the farmer. This data was not available and therefore the sensitivity analysis of adoption of pesticides is not conclusive. Since BC acts as a substitute to pesticides, increase in use of pesticides will lead to a reduction in benefits to BC program when economic benefits were confined to yield loss abated. It is, however, highly doubtful that in the foreseeable future farmers in the area will adopt the purchased inputs even at low levels owing to the high poverty and low education level.

Net present value would increase by 7.9% if yield loss abated increases by 10% beyond the projected level after 2005. If the interest rate reduces to 5% the NPV will increase by 17%. A 10% increase in prices after 2005 will lead to a 7% increase in NPV. Increasing the period of analysis to 30 years, and assuming that the parasitoid will cause stem borer density to stabilize at an effective density of 1.1, the economic gains would increase by 170%. These results show that under all circumstances, the BC project will still be profitable.

Conclusion

The IRR and benefits cost ratios show that investment in the stem borer biological control program gave high returns to investment. With these benefits, the project can be rated as one of the successful projects in biological control in Africa that has direct impact on the local community since maize is a staple food in all households. The stream of economic benefits is expected to accrue in perpetuity since the parasitoid has permanently been established in the ecosystem.

The greatest achievement by the project has been the suppression of the stem borer populations, which were still in the increase. From the analysis, parasitism by the introduced parasitoid is still growing and pest densities are expected to continue decreasing. It can be expected that other parasitoid species targeting the egg and pupal stages of the stem borer life cycle

will speed up pest suppression and push yield losses by stem borers to an insignificant level. Thus, the exotic solitary braconid pupal parasitoid *Xanthopimpla stemmator* (Thunberg 1822) was imported by ICIPE in 2000 for classical biological control of *Chilo partellus* and released in Kenya in 2005. *X. stemmator* has successfully established on *Chilo sacchariphagus* (Bojer 1856) (Lepidoptera: Crambidae) in sugarcane fields (Conlong & Goebel 2002) and recently on *C. partellus* on maize in Mozambique (Cugala *in lit.*). Previous laboratory work by Gitau (2002) indicated that this endoparasitoid would attack and develop in *C. partellus*, *B. fusca* and the noctuid *S. calamistis*, thus, it might also reduce total borer densities in the area afflicted.

References

Ajala S.O., Saxena K.N. 1994. Interrelationship among *Chilo partellus* Damage Parameters and their Contribution to Grain Yield Reduction in Maize (*Zea Mays* L.). *Applied Entomology and Zoology* 29(4): 469-476.

Ajala S.O., Kling J.G., Schulthess F., Cardwell K., Odiyi A. 2003. Progress in breeding for resistance to maize stem borers *Sesamia calamistis* and *Eldana saccharina* in West and Central Africa. *Proceeding from the 7th Eastern and Southern Africa Regional Maize Workshop, 2002*: 49-54.

Baker L.J. 2000. *Evaluating the impact of development projects on poverty. A handbook for practitioners.* The World Bank. Washington DC. 217 p.

Bokonon-Ganta A., De Groote H., Neuenschwander P. 2002. Socio-economic impact of biological control of mango mealybug in Benin. *Agriculture, Ecosystems and Environment* 93: 367-378.

Borgemeister C., Chabi-Olaye A., Nolte C., Schulthess F. 2005. Abundance, dispersion and parasitism of the stem borer *Busseola fusca* (Lepidoptera: Noctuidae) in maize in the humid forest zone of southern Cameroon. *Bulletin of Entomological Research* 95: 169-177.

Bosque-Pérez N.A., Mareck J.H. 1991. Effect of the stem borer *Eldana saccharina* (Lepidoptera: Pyralidae) on the yield of maize. *Bulletin of Entomological Research* 81: 243-247.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005a. Effects of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller) Lepidoptera: Noctuidae) in the humid forest of southern Cameroon. *Agriculture, Ecosystem and Environment* 108: 17-28.

Chabi-Olaye A., Nolte C., Schulthess F., Borgemeister C. 2005b. Relationships of maize intercropped and stem borer damage to maize yield and land-use efficiency in the humid forest of Cameroon. *Bulletin of Entomological Research* 95: 417-427.

Chabi-Olaye A. Nolte C., Schulthess F. Borgemeister C. 2005c. Abundance, dispersion and parasitism of the noctuid stem borer *Busseola fusca* (Fuller) in mono- and intercropped maize in the humid forest zone of southern Cameroon. *Bulletin of Entomological Research* 95: 169-177.

CIA 2005. *The world fact book.* CIA, Washington DC, USA.

CIBC 1968-72. Annual reports of the Commonwealth Institute of Biological Control.

Colong D.E., Goebel F.R. 2002. Biological control of *Chilo sacchariphagus* (Lepidoptera: Crambidae) in Mocambique: the first steps. *Proceedings of South African Sugar Technologist Association* 76: 710-720.

De Groote H., Bett C., Okuru O. J, Odendo M., Mose L., Wekesa E. 2001. Direct estimation of maize crop losses due to stem borers in Kenya, preliminary results from 2000 and 2001. *Proceeding of the Seventh Eastern and Southern Africa regional maize conference* : 401-406.

De Groote H., Overholt W.A., Ouma J.O., Mugo S. 2003a. Assessing the impact of Bt maize in Kenya using GIS model. *International Agricultural Economics Conference*, Durban, August, 2003.

De Groote H., Ajuonu O., Attignon S., Djessou R., Neuenschwander P. 2003b. Economic impact of biological control of water hyacinth in Southern Benin. *Ecological Economics* 45: 105-117.

FAO 1995. *FAO production yearbook 1995*. Food and Agricultural Organization of the United Nations, Rome.

Freeman H.A., Coe R. 2002. Smallholder farmers' use of integrated nutrient management strategies: Patterns and possibilities in Machakos Disttict of Eastern Kenya, p. 143-54 *in*: **Barrett C.B., Place F., Aboud A.A. (eds.)**, *Natural Resources Management in African Agriculture: Understanding and Improving Current Practices*. CAB International, Wallingford. 368 p.

Gitau A.C.W. 2002. *Development of pupal parasitoid* Xanthopimpla stemmator *(Thunberg) (Hymenoptera: Ichneumonidae) in various cereal stem borers (Lepidoptera)*. Msc Thesis, Kenyatta University.

Gohole L.S., Overholt W.A., Khan Z.R., Vet L.E.M. 2003. Role of volatiles emitted by host and non-host plants in the foraging behaviour of *Dentichasmias busseolae*, a pupal parasitoid of the spotted stem borer *Chilo partellus*. *Entomologia Experimentalis et Applicata* 107: 1-9.

Gounou S., Schulthess F., Shanower T., Hammond W.H.O., Braima H., Cudjoe A.R., Adklaoe R., Antwi K.K., Olaleye I. 1994. *Stem and ear borers of maize in Ghana, Plant Health Management Research Monograph 4*. International Institute of Tropical Agriculture, Ibadan, Nigeria, 23p.

Hassan R.M. 1998. *Maize technology development and transfer: A GIS application for research planning in Kenya*. CAB International/CIMMYT/KARI, Wallingford. 256 p.

Horst W.J., Härdter R. 1994. Rotation of maize with cowpea improves yield nutrient use of maize compared to maize monocropping in an alfisol in the northern Guinea Savanna of Ghana. *Plant and Soil* 160: 171-183.

Huffaker C.B., Simmonds F.J., Laing J.E. 1976. Theoretical and empirical basis of biological control, p. 41-78. *in*: **C. B. Huffaker, Messenger P. S. (eds.)**, *Theory and practice of biological control*. Academic press, New York.

Jiang N., Schulthess F. 2005. The effect of nitrogen fertilizer to maize on the bionomics of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and performance of its larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). *Bulletin of Entomological Research* (*in press*).

Jiang N. Zhou, G. Overholt W.A., Muchugu E., Schulthess F. 2006. The temporal correlation and spatial synchrony in the stem borer and parasitoid system of coastal Kenya with climate effects. *Annales de la Société Entomologique de France* 42(3-4): 381-387.

Kfir R. 1994. Attempts at biological control of the stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in South Africa. *African entomology* 2: 67-68.

La Croix E.A.S. 1967. Maize stalk borers in the coast province of Kenya. *East African Agriculture and Forestry Journal* 17: 61-68.

Macharia I., Lohr B., De Groote H. 2005. Assessing the potential impact of biological control of *Plutella xylostella* (diamondback moth) in cabbage production in Kenya. *Crop Protection* (*in press*).

Mailu A. 1997. *Review of Kenyan agricultural research. Pests of Plants*. KARI, Nairobi, Kenya, Vol. 29.

Mathez F.C. 1972. *Chilo partellus* Swinh., *C. orichalcociliella* Strand (Lep., Crambidae) and *Sesamia calamistis* Hmps. (Lep., Noctuidae) on maize in the Coast Province, Kenya. *Mitteilungender der Schweizerischen Entomologischen Gesellschaft* 45: 267-289.

Ndemah R., Schulthess F. 2002. Yield of maize in relation to natural field infestation and damage by lepidopteran borers in the forest and forest/savanna transitional zones of Cameroon. *Insect Science and its Application* 22: 183-192.

Norgaard R.B. 1988. The biological control of the cassava mealy bug in Africa. *American Journal of Agricultural Economics* 70: 366-371.

Ofomata V.C., Overholt W.A., Lux S.A., Huis A., Egwuatu R.I. 2000. Comparative studies on the fecundity, egg survival, larval feeding, and development of *Chilo partellus* and *Chilo orichalcociliellus* (Lepidoptera: Crambidae) on five grasses. *Annals of the Entomological Society of America* 93: 492-499.

Omamo S.W. 1998. Transport costs and smallholder cropping choices: an application to Siaya District, Kenya. *American Journal of Agricultural Economics* 80: 116-23.

Omwega C.O., Kimani S.W., Overholt W.A., Ogol C.K.P.O. 1995. Evidence of the establishment of *Cotesia flavipes* (Hymenoptera: Braconidae) in continental Africa. *Bulletin of Entomological Research* 85: 525-530.

Omwega C.O., Overholt W.A., Mbapila J., Kimani-Njogu S.W. 1997. Establishment and dispersal of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), and exotic endoparasitoid of *Chilo partellus* Swinhoe (Lipidoptera: Pyralidae) in Northern Tanzania. *African Entomology* 5: 71-75.

Omwega C.O., Muchugu E., Overholt W.A., Schulthess F. 2006. Release and establishment of *Cotesia flavipes* (Hym., Braconidae) an exotic parasitoid of *Chilo partellus* (Lep., Cambridae) in East and Southern Africa. *Annales de la Société Entomologique de France* 42(3-4): 511-517.

Overholt W.A., Ngi-Song A.J., Omwega C.O., Kimani-Njogu S.W., Mbapila J., Sallam M.N., Ofomata V. 1997. A review of the introduction and establishment of *Cotesia flavipes* Cameron in East Africa for biological control of cereal stem borers. *Insect Science and its Application* 17: 79-88.

Owuor J. 2002. *Determinants of Agricultural production in Kenya*. Tegemeo institute of Agricultural policy and development/ Egerton University.

Pielou, E.C. 1977. *Mathematical Ecology*. John Wiley & Sons, Inc. USA.

Republic of Kenya 1995-2004. District Annual Reports. Government of Kenya.

Schulthess F., Chabi-Olaye A., Gounou S. 2004. Multi-trophic level interactions in a cassava –maize mixed cropping system in the humid tropics of West Africa. *Bulletin of Entomological Research* 94: 261-272.

Schulthess F., Bosque-Pérez N.A., Chabi-Olaye A., Gounou S., Ndemah R., Goergen. G. 1997. Exchanging natural enemies species of lepidopterous cereal stem borers between African regions. *Insect Science and its Application* 17: 97-108.

Seshu Reddy K.V., Walker P.T. 1990. A review of the yield losses in graminaceous crops caused by *Chilo* spp. *Insect Science and its Application* 11: 563-569.

Seshu Reddy K.V., Sum K.O.S. 1991. Determination of economic injury of stem borer, *Chilo partellus* in Maize, *Zea mays* L. *Insect Science and its Application* 12: 269-274.

Sétamou M., Schulthess F. 1995. The influence of egg parasitoids belonging to the *Telenomus busseolae* (Hym.: Scelionidae) species complex on *Sesamia calamistis* (Lepidoptera: Noctuidae) populations in maize fields in southern Benin. *Biocontrol Science and Technology* 5: 69-81.

Sétamou M., Schulthess F., Bosque-Pérez N.A., Thomas-Odjo A. 1995. The effect of stem and cob borers on maize subjected to different nitrogen treatments with special reference to *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Entomologia Experimentalis et Applicata* 77: 205-210.

Sétamou M., Cardwell K., Schulthess F., Hell K. 1998. Effect of insect damage to maize ears, with special reference to *Mussidia nigrivenella* (Lepidoptera; Pyralidae), on *Aspergillus flavus* (Deuteromycetes; Monoliales) infection and alfatoxin production in maize before harvest in the Republic of Benin. *Journal of Economic Entomology* 91: 433-438.

Sétamou M., Nanqing J., Schulthess F. 2005 Effect of the host plant on the survivorship of parasitized *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) larvae and performance of its larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). *Biological Control* 32: 183-190.

Shanower T., Schulthess F., Bosque-Pérez N.A. 1995. The effect of larval diet on the growth and development of *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Insect Science and its Application* 14: 681-685.

Tams W.H.T. 1932. New species of African Heterocera. *Entomologist* 65: 1241-1249.

Turner P.C., Sylla A., Gong Y.Y., Diallo M.S., Sutcliffe A.E., Hall A.J., Wild C.P. 2005. Reduction in exposure to carcinogenic aflatoxins by postharvest intervention in West Africa: a community-based intervention study. *Lancet* 365: 1950-56.

Usua E.J. 1968. Effect of varying populations of *Busseola fusca* larvae on the growth and yield of maize. *Journal of Economic Entomology* 61: 375-376.

Wekesa E., Mwangi W., Verkuijl H., Danda K., De Groote H. 2003. *Adoption of maize production technologies in the coastal lowlands of Kenya.* CIMMYT, Mexico, D.F.

Zhou G., Overholt W.A. 2001. Spatial-Temporal Population Dynamics of *Cotesia flavipes* (Hymenoptera: Braconidae) in Kenya. *Environmental Entomology* 30: 869-876.

Zhou G., Baumagartner J., Overholt W.A. 2001. Impact assessment of an exotic parasitoid on stem borer (Lepidoptera) population dynamics in Kenya. *Ecological Applications* 11: 1554-1562.

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Langue. Les travaux sont en anglais (U.K.) ou en français (France).

Présentation du manuscrit. Les manuscrits doivent être divisés en sections bien définies : Introduction, Matériel et méthodes, Résultats (ou Observations), Discussion, Conclusion (facultatif), Remerciements, Références. La taxonomie doit se conformer aux recommandations de la dernière version du Code International de Nomenclature Zoologique. Le nom de l'auteur et la date de description doivent être fournis pour chaque taxon à sa première citation comme dans les exemples suivants: *Bombus terrestris* (L. 1758); *Hoplitis parvula* (Dufour & Perris 1840). Dans le manuscrit, le symbole mâle doit être remplacé par $ ou $$ (au pluriel), le symbole femelle doit être remplacé par £ ou ££ (au pluriel). Les dates doivent être conformes aux exemples suivants: 13.IV.1957; 31.VIII.2003; 2.II.2004.

Mise en page. Les pages sont au format DIN A4. Le texte, les références, les tables et les légendes doivent être en double espacement, avec des marges supérieure, inférieure et latérales de 2,5 cm. Toutes les pages doivent être numérotées. Le texte doit être en Times (New Roman) 12pt, justifié à gauche, sans indentation. Les noms de genres, espèces et sous-espèces doivent être en italique.

Tables. Les tables doivent être sur des pages séparées après les Références, avec la légende au-dessus. Le format des tables doit correspondre à la justification des annales en mode «portait» (1 ou 2 colonnes: 8 ou 16,5 x 22,8 cm).

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Références. Les citations doivent être conformes aux exemples suivants: «Paulian affirme que... (Paulian 1988)»; «Comme indiqué par Paulian (1988)»; «(Regier & Schultz 1998: 331)»; «(Bartels 1983; Paulian 1988; Bitsch 2001)»; «(Bontems 1988; Jamet *et al.* 1999)». Les références doivent être conformes aux exemples ci-dessous.

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Language. Papers are in English (U.K.) or in French (France).

Manuscript layout. Manuscripts should be divided into precise sections: Introduction, Material and methods, Results (or Observations), Discussion, Conclusion (optional), Acknowledgements, References. The author name and description date must be given for each taxon at its first mention as in the next examples: *Bombus terrestris* (L. 1758); *Hoplitis parvula* (Dufour & Perris 1840). In the manuscript, the male symbol must be

replaced by $ or $$ (plural), the female symbol must be replaced by £ or ££ (plural). The dates should be given as in the following examples: 13.IV.1957; 31.VIII.2003; 2.II.2004.

Page layout. The page format is DIN A4. The text, as well as references, tables and legends, should be double spaced with upper, lower and lateral margins of 2.5cm. All pages must be numbered. The text should be in Times (New Roman) 12pt, left justified, without indentation. Names of genera, species and subspecies must be in italics.

Tables. The tables be provided on separate pages after the References, with the legend at the top. The table format must fit the *Annales* "portait" justification (1 or 2 columns: 8 or 16.5 x 22.8 cm).

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Exemples de références / *References sample*

Livre / *Book*:

Paulian R. 1988. *Biologie des Coléoptères*. Lechevalier, Paris, 719 p.

Thèse / *Thesis*:

Bartels P.J. 1983. *Polygyny and the reproductive biology of the Argentine ant*. Ph. D. Dissertation, University of California, Santa Cruz, 205 p.

Dupont R. 1988. *Phylogénie des Hyménoptères Figitidae*. Thèse de doctorat, Université de Liège, Liège, 307 p.

Partie d'un travail collectif / *Part of a collective publication*:

Bitsch J. 2001. The hexapod appendage: basic structure, development, and origin, p. 175-193 *in*: **Deuve T. (ed.)**, *Origin of the Hexapoda. Annales de la Société entomologique de France* (n.s.) **37**: 1-304.

Bontems C. 1988. Localization of spermatozoa inside viviparous and oviparous females of Chrysomelinae, p. 299-316 *in*: **Jolivet P., Petitpierre E., Hsiao T. H. (eds.)**, *Biology of Chrysomelidae*. Kluwer, Dordrecht.

Livre inclu dans une collection numérotée / *Part of a book serial*:

Brindle A. 1969. *Insectes Dermaptères. Faune de Madagascar*, **30**, ORSTOM-CNRS, Paris, 112 p.

Comptes-rendus / *Proceedings*:

Medvedev L.N. 1971. The ways of evolution and phylogeny of Chrysomelidae (Coleoptera). *Proceedings of the 13th International Congress of Entomology* **1**: 271.

Article dans une revue / *Paper in a serial*:

Danks H.V. 1971. Biology of some stem-nesting aculeate Hymenoptera. *Transactions of the Royal entomological Society of London* **122** [1970]: 329-399.

Article avec plusieurs auteurs / *Paper with several authors*:

Jamet C., Waller A., Albouy V., Caussanel C. 1999. Description d'espèces nouvelles de Dermaptères de Madagascar et mise à jour d'une liste de Dermaptères malgaches. *Annales de la Société entomologique de France* (n.s.) **35**: 71-90.

Regier J.C., Schultz J.W. 1998. Molecular phylogeny of arthropods and significance of the Cambrian "explosion" for molecular systematics. *Molecular Biology and Evolution* **14**: 902-913.

SOMMAIRE / CONTENTS