Bean Pest Management in East Africa -

A scientific evaluation of organic insect control practices used by Tanzanian farmers



A Dissertation submitted to the Swiss Federal Institute of Technology for the Degree of Doctor of Natural Sciences

presented by

Ursula Verena Paul Dipl. Ing. Agr. ETH born June 6th, 1967 from Zürich (ZH) Accepted on recommendation of Prof. Dr. Peter J. Edwards, examiner Prof. Dr. Alex Widmer, co-examiner Dr. Angelika Hilbeck, co-examiner Dr. Urs Schaffner, co-examiner Diss ETH No. 17528

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Picture on front cover:

Map of Africa, with darker shades representing higher population density, and each black dot representing 500ha of field beans. Bean cultivars are shown in the background (in areas of lowest population density).

Adapted from:

Wortmann CS, Kirkby RA, Eledu CA, Allen DJ. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa, CIAT – Pan-Africa Bean Research Alliance, 1998.

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Beans have been introduced to Africa more than 400 years ago. Since then they became the second most important source of protein and third most important source of energy for the African people. The pest complex is characterised by a combination of endemic insects as well as seed borne cosmopolitan insects. Over time, African farmers developed pest management strategies adapted to their situation. With intensification, some of those strategies became insufficient and new approaches are sought after. This dissertation explores local knowledge and evaluates effectiveness of selected practices throughout the production cycle. It combines research in close collaboration with farmers with research on station and in the laboratory.

An endemic field pest, *Ootheca bennigseni*, eats the young bean leaves, before it oviposits its eggs close to bean roots, where the larvae feed and develop. Farmers want a field spray to reduce the leaf damage and the resulting yield loss. As industrial insecticides are too expensive and often unavailable, they suggest using local substances such as an extract of *Vernonia lasiopus* (vernonia) and organic substances such as cow urine. In a researcher managed trial on a farmer's field, three applications of an aqueous extract of vernonia, diluted cow urine, and two controls (water and lambda cyhalothrin) were applied. Insect abundance of adults and larvae were measured and leaf damage assessed. Cow urine proved to be highly effective in reducing adult insect abundance for at least 24 hours. In comparison, Vernonia reduced adult abundance less effectively but effects lasted for at least seven days. Leaf damage was significantly reduced by application of vernonia during the peak infestation period, but urine treated plants were not less damaged than control plants, which shows that the frequency of the treatments was not sufficient. Larvae abundance assessed at harvest time and yield were not

improved in any of the treatments including lambda cyhalothrin, which controlled the adult abundance successfully and reduced leaf damage significantly.

The cosmopolitan insect *Acanthoscelides obtectus* is mainly known as a storage pest. But it is also a field pest in its own right and infests beans while they ripe in the field. Several field and laboratory trials were conducted to establish pre-harvest infestation preferences. The adult insect was found in fields six weeks before harvest in research fields, but only one week before harvest in farmers' fields. Pods from fields close to homesteads were more often infested than those from fields at least one kilometre away from habitation or storage facilities. Amongst the pods collected from farms, only pods at the end of wilting stage or drier were infested by *A. obtectus*. Delayed harvest increased infestation in dry bean pods. In laboratory no-choice trials, pods at physiological maturity or maturer were infested similarly. However, when given the choice, the insect preferred the maturer pods. Infestation rates did not differ between open or closed pods. Dry mature pods stimulated oviposition more than an empty dish (no pod and no bean seed), but it stimulated oviposition less than the seeds alone or the complete pod with beans seeds.

Storage losses are mainly due to a pest complex of two insects: *Acanthoscelides obtectus* and *Zabrotes subfasciatus*. Farmers traditionally use dried botanicals for controlling storage pests in beans. Some traditional botanicals and some other locally available plants were tested against both bruchids on farm and in the laboratory. In laboratory trials, *Chenopodium ambosoides* was most effective with an insect mortality of 100% in less than three days for *A. obtectus* and *Z. subfasciatus*. Powdered *Tagetes minuta* increased mortality significantly more than no botanical or powdered bean leaves. Entire *T. minuta* leaves did not increase mortality, nor did *Cupressus lusitanica* or *Azadirachta indica* or bean leaves in either powder or leaf form. In on farm trials, *A. indica* seed powder was the most effective treatment. The on-farm trials suggested that *A. indica* seed powder is effective in protecting stored products for up to four months (or for two to three generations of insects). However, *C. ambrosioides* and *T. minuta* (both dried and ground young plants) and to a lesser degree *C. lusitanica* (leaves in powdered form) also have a good potential for short term storage (up to two months or one to two generations of insects).

In the synthesis five theses on farmers' pest management are discussed in the light of the author's personal experiences.

- (1) Farmers use treatments that control pests, but effectiveness and duration of control varies greatly.
- (2) Farmers concentrate their pest management efforts to where it is most effective: more control practices are used in storage than in the field crop.
- (3) Farmers observe, experiment, and adapt production and storage with respect to local conditions.
- (4) Farmers know the damage done by pests, but their knowledge on the pest ecology is limited.
- (5) When farmers understand the lifecycle of the pest in more detail, they gain confidence and are more likely to teach other farmers about their control practices.

In conclusion this research shows the need to include farmers in learning trials. Only what they experience and see can be internalised to bring about change. It is crucial that farmers learn to understand life cycles of insects, or how diseases spread, so that they can take simple measures to reduce their losses. Bohnen wurden vor mehr als 400 Jahren nach Afrika eingeführt. Heute tragen Bohnen als zweitwichtigste Nahrungsquelle für Protein und als drittwichtigste Nahrungsquelle für Energie massgeblich zur ausgewogenen Ernährung der Afrikanischen Völker bei. Der Schädlingskomplex ist durch eine Kombination von endemischen und weltweit verbreiteten Insekten charakterisiert. Mit der Zeit entwickelten die Afrikanischen Bauern Pflanzenschutzmassnahmen, die an ihre Situation angepasst waren. Mit der Intensivierung, boten jedoch manche Massnahmen ungenügenden Schutz, und deshalb wurde nach neuen Möglichkeiten gesucht. Diese Dissertation erkundet das lokale Wissen der Bauern und beurteilt die Wirkamkeit von ausgewählten Praktiken während dem ganzen Anbauzyklus. Diese Studie verbindet Forschung in enger Zusammenarbeit mit den Bauern mit Forschung auf der Versuchsstation und im Labor.

Ein endemischer Schädling der Bohnenpflanze, *Ootheca bennigseni*, frisst an den jungen Bohnenblättern, bevor er seine Eier nahe zu Bohnenwurzeln legt, von denen sich die Larve ernährt. Die weitere Entwicklung findet im Boden statt. Bauern erwünschen sich ein Spritzmittel, um den Blattschaden und die davon resultierende Ertragseinbusse zu vermindern. Aber da die industriellen Insektizide oft zu teuer oder nicht erhältlich sind, schlagen sie vor, locale Substanzen wie einen Extrakt von *Vernonia lasiopus* (Vernonia) oder organische Substanzen wie Kuhurin zu benutzen. Forscher führten Versuche in Feldern von Bauern durch. Dabei wurden drei Spritzungen von einem Wasserextrakt von Vernonia, verdünntem Kuhurin und zwei Kontrollen (Wasser und Lambda Cyhalothrin) durchgeführt. Adult- und Larven-abundanz wurden gemessen und der Schaden an Blättern erfasst. Kuhurin erwies sich als sehr effektiv, um die Anzahl erwachsener Insekten während 24 Stunden zu reduzieren. Vernonia im Vergleich, war weniger effektiv, dafür dauerte der Effekt für mindestens sieben Tage. Blattschaden nach Behandlung mit Vernonia war vermindert während der Hauptinfestationszeit. Aber Pflanzen, die mit Urin behandelt wurden wiesen gleich viel Schaden wie unbehandelte Pflanzen auf. Dies zeigt, dass häufiger hätte behandelt werden müssen. Larvenabundanz, zur Erntezeit, und Ertrag waren bei keiner Behandlung verbessert, auch nicht bei Lambda Cyhalothrin, das sowohl die Adultenabundanz erfolgreich kontrollierte, als auch den Blattschaden signifikant verminderte.

Das weltweit verbreitete Insekt, Acanthoscelides obtectus, ist vor allem als Lagerhaltungsschädling bekannt. Aber es ist selber auch ein Feldschädling in reifenden Bohnenfeldern. Mehrere Feld- und Laborversuche wurden durchgeführt, um dessen Vorlieben herauszufinden. Adulte wurden sechs Wochen vor Ernte in Feldern der Forschungsstation gefunden. In Feldern der Bauern, wurden die ersten Adulten erst eine Woche vor Ernte gefangen. Hülsen von Feldern, die näher als einen Kilometer von Häusern enfernt waren, waren häufiger befallen, als Hülsen von Feldern, die weiter enfernt waren. Auf Feldern der Bauern, wurden nur Hülsen befallen, die reifer als als das Welkstadium waren. Verzögerte Ernte führten zu erhöhtem Befall durch A. obtectus. In Laborversuchen, bei denen das Insekt keine Auswahl zwischen mehreren Hülsen hatte, wurden die Hülsen aller Reifestadien ähnlich häufig befallen. Wenn das Insekt jedoch die Wahl zwischen zwei Hülsen hatte, bevorzugte es die reifere. Ob die Hülsen offen or geschlossen waren, machte keinen Einfluss auf die Befallsrate. Ovipositionstimulation war höher bei trockenen, reifen Hülsen, als bei weniger reifen. Die Hülse alleine (ohne Samen) stimuliert Oviposition in A. obtectus mehr als eine leere Petrischale (ohne Hülse, ohne Samen), aber die Hülse allein stimulierte Oviposition weniger als entweder ganze mit Samen gefüllte Hülsen oder Bohnensamen alleine.

Verluste bei der Lagerhaltung werden vor allem von einem Schädlingskomplex von zwei Insekten verursacht: *Acanthoscelides obtectus* und *Zabrotes subfasciatus*. Traditionellerweise gebrauchen die Bauern getrocknete Pflanzen, um die Lagerhaltungsschädlinge in Bohnen zu kontrollieren. Traditionelle und andere örtlich erhältliche Pflanzen wurden im Labor und bei Bauern auf Ihre Wirkasamkeit gegen beide Schädlinge getestet. In Laborversuchen, war *Chenopodium ambrosioides* am wirkungsvollsten. Die Mortalität war 100 % in weniger als drei Tagen für beide Arten. Pulver von *Tagetes minuta* erhöhte die Mortalität siginifikant, verglichen mit keinem

Pflanzenmaterial oder mit gepulverten Bohneblättern. Unzerkleinerte Blätter von *T. minuta* hatten keine Wirkung auf die Mortalität. Sowohl Blätter von *Cupressus lusitanica* und *Azadirachta indica* waren unabhängig von der Anwendungsform wirkungslos. Bei Versuchen mit Bauern war das Samenpulver von *A. indica* am wirkungsvollsten. Der Versuch schlägt eine Wirkungsdauer von ungefähr vier Monaten (oder zwei bis drei Insektengenerationen) vor. Jedoch sowohl *C. ambroisioides* und *T. minuta* (beider Blätter als Pulver verwendet), als auch in geringeren Massen *C. lusitanica* zeigen ein hohes Potential zur kurzzeitigen Lagerhaltung (bis zwei Monate oder eine Generation der Schädlinge).

In der Synthese werden fünf Thesen betreffend der Schädlingsbekämpfung durch die Bauern aus persönlicher Erfahrung diskutiert.

- (1) Bauern benützen Schädlingsbekämpfungsmassnahmen, aber deren Effizienz und Wirkungsdauer unterscheiden sich erheblich.
- (2) Bauern benützen Schädlingsbekämpfungsmassnahmen vor allem dort, wo es am effizientesten ist: In der Lagerhaltung werden mehr Massnahmen durchgeführt, als im Feldanbau.
- (3) Bauern beobachten, experimentieren und passen ihre Anbau- und Lagerhaltungsmethoden an lokale Umstände an.
- (4) Bauern kennen den durch Schädlinge verursachten Schaden, aber ihr Wissen der Insektenökologie ist beschränkt.
- (5) Wenn die Bauern den Lebenslauf der Insekten besser verstehen, gewinnen sie mehr Selbstbewusstsein und geben ihr Wissen eher an andere Bauern weiter.

Abschliessend hat diese Forschung gezeigt, dass es wichtig ist, Bauern in Lernversuche einzubeziehen. Nur wenn sie selbst mitmachen und das Insekt sehen, kann das neue Wissen einverleibt werden. Es ist paramount, dass Bauern die Lebensläufe der Insekten verstehen lernen, oder dass sie die Vermehrung von Krankheiten verstehen, damit sie in der Lage sind, mit einfachen Massnahmen ihre Verluste zu vermindern.

In Sub-Saharan Africa, agriculture is the most important enterprise and the key to economic development. It is characterised by a large number of smallholdings of no more than one happen household (Abate et al. 2000). Most farmers are resource poor in terms of access to natural resources, credit, information and external inputs. Their main objective is subsistence by necessity, and they farm with predominantly traditional methods (Van Huis & Meerman, 1997). Beans (*Phaseolus vulgaris* L.) form an important food and cash crop in Africa, particularly in the Eastern, Southern and Great Lakes regions of the continent (Abate & Ampofo, 1996). Of all commodities produced in these parts of Africa, beans are considered the second most important source of human dietary protein (after maize) and the third most important source of calories (after maize and cassava) (Pachico, 1993). Beans are also a major source of iron and calcium (Shellie-Dessert & Bliss, 1991). Beans originated in the highlands of Central and South America and were introduced into Africa some 400 years ago (Greenway, 1945). Beans became established as a food crop in Africa before the colonial era, but there is little indication of the status the crop attained. The wealth of local names given to distinctive cultivars, and the genetic variation, are together evidence of the long establishment of beans as a crop (Wortmann & Allen, 1994). Because the crop arrived without many of its field pests, the pest spectrum for beans in Africa differs significantly from that attacking the crop in its ancestral region. Therefore the knowledge base on those pests is relatively small. The major exceptions are storage pests, which tend to be seed borne and therefore are cosmopolitan in their distribution. However, many indigenous pests of other legumes, notably the cow pea, Vigna spp. and its close relatives (other Leguminosae), have adapted to the crop, and every part of the bean plant – from roots to the mature pods - is attacked (Abate & Ampofo, 1996). Therefore, pest management is an integral and crucial component of bean production in East Africa. Crop protection aspects of traditional agriculture have evolved with the system and

are complex (Bajwa & Schaefers, not dated). These low input systems often operated efficiently, but generally did not produce high yields (Van Huis & Meerman, 1997). On the positive side, pest outbreaks in these conditions were rare. However increasing population pressures are changing this situation rapidly and pest problems are expected to continually increase (Abate et al, 2000). The feared food deficits in tropical developing countries have compelled national programs and international donors to place a high priority on improving the agricultural productivity and the economic well-being of the small scale farmer (Matteson et al, 1984). However in Africa, most efforts, including the promotion of the so called "Integrated Pest Management (IPM)" approach, experience difficulties in uptake by resource poor farmers (Orr & Ritchie, 2004), and success is limited to some cash crops (Van Huis & Meerman, 1997). The reasons are inadequate understanding of indigenous crop protection practices, local ecology, and socio-economic factors involved in any modification of the existing production system (Bajwa & Schaefers, not dated). Therefore, new approaches such as on-farm cropping systems research (Matteson et al, 1984), farmer-first (FF) (Chambers, 1991), farmer field schools (FFS), local agricultural research committees (CIAL) (Braun et al, 2000), farmer participatory research (FPR) (Williamson, 1999) and participatory technology development (PTD) (Veldhuizen et al, 1997) have been suggested. The common feature of all these approaches is a purposeful interaction between rural people and outside facilitator leading to best-bet options integrating research findings with local knowledge, and taking into account specific practices and socio-economic aspects. This also asks for a better collaboration between social and natural scientists. There are success stories and lessons about failures reported (Orr & Ritchie, 2004), but only time will tell us if those approaches lead to improved livelihoods in wider areas. This new paradigm of interaction with farmers and appreciation of local knowledge led to many studies on traditional pest management. But still, there is a remarkable deficit on literature testing the efficacy of traditional pest management practices with the exception of the well documented storage methods and practices.

This dissertation grew out of a project on dissemination and adoption of complex agricultural technologies using an action research framework based on participatory learning and action (PLA) (Pretty et al. 1995). During this research, farmers' priority problems in agriculture were investigated, local management strategies explored

and tested. Learning trials as well as technology adaptation trials were conducted to find locally acceptable solutions to those problems (Hollenweger & Ampofo, 2001, Hollenweger & Mkalimoto, 2001). All trials yielded valuable learning to the farmers and the researchers, but especially local practices needed a more in depth evaluation, before any recommendation could be made. Therefore, parallel trials on the research station and/or farmers' fields were conducted. Researchers managed them as closely as possible to on-farm situations. They included treatments used or suggested by farmers, and were designed to verify, quantify and evaluate the results of those organic insect control practices. The central part of this thesis reports the results from these trials. They include trials on control strategies against an early field pest (*Ootheca bennigseni* Weise), a pest at the borderline between field and storage (*Acanthoscelides obtectus* Say) and a storage pest complex (*A. obtectus* and *Zabrotes subfasciatus* Boheman). Hence this dissertation covers the entire bean production cycle from planting to storage with some of the most important pests occurring in Tanzania.

Chapter four reports on tests using cow urine and *Vernonia lasiopus* var. *iodocalyx* (O. Hoffmann), a local medicinal herb, against an early field pest in beans, *Ootheca bennigseni* (Weise).

O. bennigseni is a chrysomelid beetle endemic to mainland Africa. Its biology has not been studied in detail, but Schneider (2002) and the entomology group at the Selian Agricultural Research Institute (SARI) and the International Centre for Tropical Agriculture (CIAT, J.K.O. Ampofo, unpubl. data) found that the oval, about 7 mm long, shiny beetle oviposits up to eight egg batches of approximately 60 eggs/batch into the soil close to bean plants. Larvae emerge after two to three weeks and feed on the roots of beans. The larvae go through three instars, which last 5 to 11 weeks each, before they pupate in an earthen cell within the soil. The teneral adults undergo a diapause until the onset of the following year's rainy season, when they emerge and start feeding on leaves of the newly planted beans. The adult beetle can cause extensive defoliation, and, with heavy infestation, may completely destroy a crop. Additionally, the feeding of the larvae on lateral roots causes wilting and premature senescence in bean plants. Yield losses of 18 – 31% are reported in Tanzania, but do not take larval damage into account (Karel & Rweyemanu, 1984). Farmers were largely unaware of the insect's lifecycle, but after

being taught cultural methods to disrupt the lifecycle, many were unable to implement those methods and searched for affordable spraying regimes with local organic substances. Farmers' trials were complemented with the reported researcher managed on-farm trial. Adult abundance, leaf damage, larval abundance and yield were measured to evaluate effectiveness and duration of effectiveness of the treatments compared to no control and an industrial insecticide.

Chapter five investigates pre-harvest infestation of beans by Acanthoscelides *obtectus* (Say) to explore if early harvest could reduce infestation by storage pests. A. obtectus and Zabrotes subfasciatus (Boheman) are the most important storage pests in Africa (Giga et al, 1992). They occur together, have a similar biology, but differ in ways of infestation: Z. subfasciatus infests threshed beans, but not whole bean pods. The adult female glues its eggs onto the bean seed, and the larva bores straight into the seed (Abate & Ampofo, 1996). In contrast A. obtectus may infests growing pods by chewing an opening into the suture and laying an egg cluster into the pod cavity (Zachariae, 1958). Hatched larvae of A. obtectus wander among the beans before penetrating (Parsons & Credland, 2003). The newly emerged adult A. obtectus mates within the first 24 hours after emergence and starts ovipositing during the next 24 hours (Parsons & Credland, 2003). The adult may feed on sugar water, but does not normally feed. It ingests pollen, but this seems to be mainly due to licking bean leave surfaces Jarry, 1987). In spite of its particular infestation pattern in the bean field, its importance as a field pest is undervalued. Most farmers are unaware of the pre-harvest infestation and do not completely understand the beetle's lifecycle, nor the origin from the damage exclusively done by the larvae.

Adult occurrence in bean fields before harvest was assessed to establish the earliest possible stage of infestation. Infestation rates in beans from different maturity, different distance from homesteads and pod characteristics were established and confirmation trials were conducted in the field with augmented pest population. Several no-choice and choice trials studied infestation preferences as well as oviposition stimulation.

Chapter six reports on laboratory and on-farm trials conducted with local botanicals to control infestation by *A. obtectus* and *Z. subfasciatus* during bean storage for up to five months. Average dry weight losses during storage have been estimated at

between 10 and 40% in average, but where management is poor, losses can be well above 50% (Kiula & Karel, 1985; Lima, 1987). Beans with multiple emergence holes of bruchid beetles and emitting a characteristic pungent odour are useless for consumption and have no commercial value (Giga et al, 1992). For this reason, the majority of farmers are forced to sell their beans at a low price immediately after the harvest for fear of damage to the crop during storage (CIAT, 1986a; Giga et al, 1992). During the field surveys in the area of study, some farmers were observed to be innovative in designing additional storage control practices (Paul & Lossini, unpublished). These included exposing beans to smoke, impregnating the storage bean sacks with hot chilly peppers or goat pellets, and mixing seed beans with kerosene or fungicides used in coffee plantations. The efficacy of such measures has not been proven and toxicity to humans could be a problem. There is a need, therefore, to investigate environmentally acceptable methods for protecting beans against bruchids during storage. Botanicals could provide an under-utilized but more effective alternative to these concoctions. And plant-derived materials have the advantages that farmers can grow them at very low costs.

This study evaluates the insecticidal properties of four botanicals under farm and laboratory conditions. Two of these - neem, *Azadirachta indica* (A. Juss), and wormseed, *Chenopodium ambrosioides* (L.) - are known in north-eastern Tanzania as medicinal plants but have not been used traditionally by farmers. The other two botanicals, cypress, *Cupressus lusitanica* var. *benthamii* (Miller) and marigold, *Tagetes minuta* (L.) are traditionally used in the area for seed storage. Many highland farmers in Arusha apply *C. lusitanica* for stored maize and beans (Paul & Mkalimoto, unpublished).

Chapter seven, the final synthesis, looks at the bigger picture of farmers' practices in bean pest management, and reports the author's own experience of working closely with farmers in pest management in Tanzania. It complements the earlier chapters with reports on processes and results of the participatory research approach.

The lessons learned are summarised in five theses on farmers' use of traditional pest control methods. Thesis one compares the efficacy and duration of control of the methods tried in the previous chapters. Thesis two discusses the discrepancy between numbers of practiced pest management methods in field crops compared

to methods used in storage. Thesis three describes how farmers experiment with pest control methods. Thesis four investigates farmers' knowledge about pests and their life cycle. In the final thesis it is demonstrated how farmers learn best about the hidden life of insects with an example of collaboration with farmers on another major bean pest, the bean fly (*Ophiomyia* spp.). This inconspicuous insect was studied together with farmers in an adaptation trial using cultural control methods. After the trial the farmers helped writing an extension leaflet to be able to share their new knowledge with other farmers. This leads to a final conclusion on dissemination and recommendations for future work to help farmers fight pest insects.

Evaluation of organic control methods of the bean beetle, *Ootheca bennigseni*, in East Africa

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ABSTRACT

Dry beans (*Phaseolus vulgaris*) are a major source of dietary protein and calories for the poor in East Africa. The increasingly abundant *Ootheca bennigseni* (Coleoptera: Chrysomelidae) is a key pest that threatens bean production and jeopardizes farmers' harvest. Participatory research with farmers suggested the need for affordable and accessible organic pest control methods. The effect of diluted cow urine and aqueous extract from vernonia (*Vernonia lasiopus* var. *iodocalyx*) leaves was evaluated in three consecutive applications. Researcher-managed on-farm trials showed that cow urine reduced pest abundance for at least 24 hours. The aqueous vernonia extract reduced the insect abundance consistently for at least seven days. Foliar damage at the peak time of infestation was significantly reduced by vernonia but not by cow urine. Future research needs to find ways to enhance and prolong the efficacy of natural substances and determine the relationship between adult abundance, larval population, and bean yield.

Keywords: *Ootheca bennigseni* Weise (Coleoptera: Chrysomelidae), *Phaseolus vulgaris* L. (Leguminosae), *Vernonia lasiopus* var. *iodocalyx* O. Hoffmann (Asteraceae), cow urine, lambda cyhalothrin.

¹ Paul UV, Ampofo JKO, Hilbeck A, Edwards PJ, 2007. Evaluation of organic control methods of the bean beetle, *Ootheca bennigseni*, in East Africa, New Zealand Plant Protection, 60:189-198.

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) contribute up to 57% of recommended dietary protein and 23% of energy to the nutrition of some African people (Shellie-Dessert & Bliss 1991). Poor people rely on a diet of beans instead of meat (Wortmann et al. 1998), and therefore it is crucial to have a secure and adequate harvest of beans.

The Selian Agricultural Research Institute (SARI) in Arusha, Tanzania, hosts a bean entomology group of the Centro Internacional de Agricultura Tropical (CIAT). In 1996, they were approached by farmers, who noticed unexplained early senescence in their beans. Investigation discovered a high larval infestation by the bean beetle (*Ootheca bennigseni* Weise (Coleoptera: Chrysomelidae)).

Ootheca bennigseni is endemic to mainland Africa and is found almost exclusively on bean plants (*Phaseolus vulgaris* L.). Its biology has not been studied in detail, but Schneider (2002) and the entomology group at the SARI and CIAT (J.K.O. Ampofo, unpubl. data) found that the oval, about 7 mm long, shiny beetle oviposits up to eight egg batches of approximately 60 eggs/batch into the soil close to bean plants. Larvae emerge after two to three weeks and feed on the roots of beans. The larvae go through three instars, which last 5 to 11 weeks each, before they pupate in an earthen cell within the soil. The teneral adults undergo a diapause until the onset of the following year's rainy season, when they emerge and start feeding on leaves of the newly planted beans. The adult beetle can cause extensive defoliation, and, with heavy infestation, may completely destroy a crop. Additionally, the feeding of the larvae on lateral roots causes wilting and premature senescence in bean plants (Abate & Ampofo, 1996). Yield losses in the range of 8-31% are attributed to O. bennigseni in Tanzania (Karel & Rweyemamu, 1984). Ootheca spp. are also reported to be a key pest in Zambia (Sithanatham, 1989), Malawi (Ross 1998), Kenya, Burundi and Rwanda (Karel & Autrique, 1989). Over the years, Tanzanian farmers have noticed increasing foliar damage by O. bennigseni to their young bean plants, but were unaware of the larval damage by the same insect until they were shown the larvae on the roots of the bean plants (Ampofo et al, 2002). Once they understood the insect's lifecycle they wanted to learn effective control methods. The scientists suggested management options based on cultural practices, but many farmers could not implement them, and requested research of traditional methods using local concoctions to be sprayed on the bean field. Treatments suggested by

farmers were fermented cow urine and an extract from vernonia (*Vernonia lasiopus* var. *iodocalyx* O. Hoffmann (Asteraceae)). On-farm trials managed by farmers showed that those treatments indeed reduced insect abundance for varying periods of time. But the farmer-collected data showed inconsistencies that severely limited the statistical analysis. Therefore a researcher-managed experiment was conducted on a farmer's field with high *O. bennigseni* incidence in the past year. The three objectives were to:

(1) determine if cow urine or vernonia extracts controlls infestation by O. bennigseni;

(2) determine the duration of the efficacy of the two treatments; and

(3) assess the treatment effect on larval abundance, foliar damage and bean yield.

METHODS

Adult O. bennigseni abundance

The experiment was conducted from April to July 2003 in a farmer's field of about 1500 m² at Tengeru/Camartec (Tanzania, Arusha region, Arumeru district, Patandi village, Duluti sub-village: 3º24'S, 36º47'E, 1205 m above sea level, mean of 1000 mm rain/year (bimodal), and a mean temperature of 21.5°C (unpublished data from a nearby flower-farm, 2002). Following the first heavy rain on 29 March, dry beans (cv. Lyamungo '91, Calima type), treated with Murtano® Dust (lindane 20%, thiram 26%) at 3 g/kg seed, were planted on 1 and 2 April. Inter-row spacing was 0.5 m and within-row spacing was 0.2 m with two seeds per planting hole. No fertilisers were applied. A randomised complete block design with six blocks was superimposed. Each block was surrounded by at least 2 m of bean plants and comprised four plots of 6×3 m, which were separated from each other by 2 m (or four rows) of beans. Each plot was subdivided perpendicular to the rows into two subplots of 3×3 m each. The natural infestation of the bean crop of this field was expected to be high, due to last year's high occurrence of *O. bennigseni*. Some adult O. bennigseni were observed on the soil surface during planting. A few volunteer plants, from last year's crop and distributed over the whole field, were left undisturbed to enhance *O. bennigseni* emergence. On 9 April, six emergence traps were placed randomly, each over a row of beans outside the trial area, and soil was put over the lower edges of the traps to close any potential escape holes between soil and frame. These traps were made of wooden frames in square pyramid form (ground surface 0.5×0.5 m and about 0.5 m high), covered with fine nylon mesh

except for a hole at the apex where a plastic bag was fixed to a metal ring to collect the emerging insects. Traps were checked daily and trapped insects were freed. The traps were removed on 29 April, as the plants filled the entire trap.

The four treatments were:

- (1) cow urine from a dairy farm in Olasiti (Arusha), which was collected in the morning into a plastic container, and left fermenting for 6±1 days in the shade at ambient temperatures. Before application, the urine was diluted with water 1:3 (v/v) to reduce the risk of burning the bean leaves.
- (2) Aqueous extract of vernonia was prepared in the evening before the day of application. Young leafy branches of vernonia were collected in Olasiti and finely ground with a wooden pestle and mortar. The ground leaves were mixed with water 1:1 (w/w), using the same method as the farmer when using botanicals, and the slurry was kept overnight in an open plastic bucket. The next morning, the mixture was strained through a fine cloth and sprayed undiluted.
- (3) The standard insecticide lambda cyhalothrin (Karate® 50 g/litre EC) was bought from a local official supplier and used at the recommended application rate of 125 mg ai/litre.
- (4) Water was used as a control.

For the three applications of treatments, a randomly chosen sub-plot of each plot was sprayed with a thoroughly cleaned knapsack sprayer between 8 and 9 am with one of the four treatments (named test sub-plot). The other adjacent sub-plot was sprayed at the same time with clean water (named control sub-plot). About 0.5±0.1 litres of liquid preparation was used for each sub-plot, which was the point where liquids started to run off. The bean plants between the trial plots were left unsprayed. The four treatments were first applied on 9 April, after counting all live O. bennigseni in all sub-plots. Counting was done with as little disturbance to the insects as possible, following each row of beans in each sub-plot. The primary leaves of most bean plants were fully opened and few O. bennigseni adults were leaffeeding. Counting was repeated 60±10 min after treatment and then daily between 9 and 10 am. It was attempted to count dead *O. bennigseni*, but this was only possible at the counting made hour after application, since a day later, the bodies had disappeared. Because of dry weather after the first application, only very few O. bennigseni emerged in the following week and the second application was delayed. A drip irrigation system was installed and an equivalent of 20 mm rain total was used between 12 and 16 April to save the young bean plants from drought damage. However, this irrigation did not result in increased insect emergence. After the next rain on 16 April (30 mm) the numbers of *O. bennigseni* increased and the bean plants developed quickly. On 21 April the field was hand weeded before the second application of the four treatments on 22 April. The bean plants had reached the two-trifoliate-leaves stage and this time 0.7 ± 0.2 litres of treatment was used for each sub-plot. The third and last application was on 30 April, at first flowering of the bean plants, and 0.9 ± 0.2 litres of treatment was used for each sub-plot. At this stage the *O. bennigseni* numbers started to drop naturally and no further applications were carried out. See Figure 1 for an overview of the time during the three applications of treatments (especially the rainfall and the irrigation) and the adult *O. bennigseni* abundance in the test sub-plots (for all four treatments).

Leaf damage

Ootheca bennigseni adults make very distinct round feeding holes predominantly on the youngest leaves of the bean plant. Therefore only one leaf damage assessment was conducted. On 6 May, 6 days after the third application, leaf damage throughout the trial period was assessed by randomly collecting three primary leaves (corresponding to first application), three trifoliate leaves from the centre of the bean plant (second to fourth trifoliate leave stage; corresponding to second application) and three trifoliate leaves from the top of the bean canopy (corresponding to third application at flowering) from each test sub-plot for damage assessment. Leaf area loss by *O. bennigseni* in percentage of total leaf area was estimated from the leaves photocopied onto graph paper and rounded to 0, 1, 5, 10, 15, 25, 50, 75 or 100% loss.

Bean yield and O. bennigseni larvae abundance

At harvest on 8 July, the beans of each test sub-plot were threshed and the grain weighed. A day later, soil samples of approximate 0.02 m³ (0.5 m long following a bean row and 0.2 m wide and 0.2 m deep) were collected from the centre of each test sub-plot. The soil was sieved (2 mm mesh) to separate and count *O. bennigseni* larvae. This method recovers about 80% of the larvae present (Ampofo & Massomo, 1998a).

Statistical analysis

For assessing insect abundance, univariate ANOVA (randomised complete block design with 4 treatments and 6 blocks, d.f.=15) was calculated for test and control sub-plots independently. The single count data were used for the analysis of abundance 1 hour after application and 1 day after application. Then the average numbers of insects on each sub-plot for the period day 1 until day 7 after application of treatments were used for an overall analysis. The LSD test was used for the separation of means. Univariate ANOVA of test sub-plot data was used for evaluation of leaf damage (average of the three leaf area loss measurements), larval counts and yield. Correlation between *O. bennigseni* abundance (average of the first seven days after each application) and the corresponding leaf area loss (average of three leaves) was calculated for each treatment separately and all data combined. Similarly, correlations between adult and larvae abundance, adult abundance and yield, larvae abundance and yield, as well as leaf area loss and yield were calculated. All correlations were calculated for the test sub-plot data only. Whenever a correlation coefficient was significant at 5% level, a regression analysis was conducted for detecting a possible difference of the regression coefficients. The software packages SPSS (version 9.2) and Microsoft Excel 2003 were used for all calculations.

RESULTS AND DISCUSSION

Adult O. bennigseni abundance

Climatic conditions and all disturbances in the bean field influenced *O. bennigseni* abundance. In particular, the abundance of *O. bennigseni* was reduced by treatment applications and heavy rainfalls as shown in Figure 1. There was also a general tendency of increasing insect abundance until end of April (before application 3), and a steady decrease afterwards. This is a typical population curve for *O. bennigseni* (Ampofo & Massomo, 1998a). The emergence traps caught an average of 2.3 *O. bennigseni* in 20 days. Although no measurements were made, it is assumed that a considerable number of beetles immigrated to the trial field from nearby bean fields, as higher insect numbers were observed at one end of the field shortly before application 2, but later the entire field seemed to be evenly infested. Few *O. bennigseni* were found after the first application of treatments, and no consistent result was obtained, therefore these data are not described. The data for each counting event of *O. bennigseni* adults are shown in Figure 1 (test sub-plots, all three

applications) and Figure 2 (control sub-plots for applications 2 and 3 only). It is apparent that all treatments had an effect on insect numbers in the test sub-plots (Fig. 1): Insects on urine treated plots dropped in the first hour after application from 40.3 to 1.5 in application 2, and from 44.0 to 9.7 in application 3. Thereafter the insect numbers increased over the next 3–4 days to similar levels as water. Vernonia treated test sub-plots revealed a less dramatic drop (15.3 to 4.8 and 34.3 to 14.2, for applications 2 and 3 respectively) and a slower recovery of the insect numbers over about 7 days. The standard lambda cyhalothrin reduced insect numbers to very low levels and kept them there for an extended period. Test sub-plots treated with the control (water) also experienced a reduction of insects 1 h after application compared to the pre-treatment count, but numbers recovered in about 1 day.



FIGURE 1: Mean adult *O. bennigseni* abundance per test sub-plot of 9 m2 under four treatments (water (control), urine, vernonia and lambda cyhalothrin (standard)), and daily irrigation (mm) and daily rainfall (mm) during three applications of the treatments.

Results from statistical analysis are summarised in Table 1. Urine treated test subplots hosted fewer *O. bennigseni* than control test sub-plots hour and day after application, but only application 3 resulted in a significant difference for the period of seven days after application. Vernonia treated test sub-plots contained significantly fewer *O. bennigseni* numbers than the control test sub-plot for 1 h, 1 d and 7 days after application 2 and 3. Lambda cyhalothrin treated test sub-plots resulted in highly significant differences for all durations and both applications. The control sub-plots reveal possible nearby effects of the treatments. While Figure 2 suggests reduced insect numbers in test sub-plots beside vernonia and lambda cyhalothrin treatments, only the lambda cyhalothrin test sub-plots contained significantly lower insect numbers than control sub-plots beside control treatments.

TABLE 1: Mean *O. bennigseni* abundance per sub-plot of 9 m² for treatment and control subplots at 1 h and 24 h after application of treatments, and for the average from day 1 until day 7. Treatments that are significantly different from the control (water) are shown by * (P<0.05) and ** (P<0.01).</p>

Time	Urine	Vernonia	Lambda	Water	LSD	LSD
			cyhalothrin	(control)	P<0.05	P<0.01
Application 2						
Test sub-plots						
1 h after application	1.5 **	4.8 **	1.0 **	17.8	9.2	12.7
24 h after application	10.2 **	5.8 **	1.0 **	31.0	14.3	19.8
7 days	27.7	12.4 *	1.6 **	35.2	19.6	27.1
Control sub-plots						
1 h after application	22.5	15.8	8.8 *	23.3	12.5	17.2
24 h after application	22.5	18.2	12.3 *	29.8	13.9	19.2
7 days	31.7	27.4	19.5 *	39.3	17.8	24.6
Application 3						
Test sub-plots						
1 h after application	9.7 **	14.2 **	1.2 **	34.3	10.3	14.2
24 h after application	3.7 **	19.5 **	6.8 **	37.2	11.7	16.2
7 days	21.5 *	21.0 **	8.3 **	27.7	4.5	6.3
Control sub-plots						
1 h after application	22.5	15.8	8.8	23.3	5.2	21.0
24 h after application	22.5	18.2	2.3**	29.8	6.8	23.2
7 days	31.7	27.4	9.5 **	39.3	6.7	9.2

It has been concluded that vernonia, urine and lambda cyhalothrin are effective at reducing O. bennigseni abundance between 1 and at least 7 days. Although urine is highly effective in reducing abundance directly after the application, it quickly loses this strong effect. The effect of vernonia lasts at least 7 days. Lambda cyhalothrin, a commercial insecticide, had a fast knock down effect, which was demonstrated by



FIGURE 2: Mean adult *O. bennigseni* abundance per control sub-plot of 9 m2 adjacent to the four treatments (water (control), urine, vernonia and lambda cyhalothrin (standard)) for applications two and three.

the discovery of dead beetles 1 h after application in these test sub-plots. It reduced insect abundance highly significantly for at least 7 days. Lambda cyhalothrin is insecticidal and known to have a repellent effect (Anon. 2000). No dead insects were discovered in urine or vernonia test sub-plots, which could either mean that there was no toxic effect or that it was slow acting (insects hide in soil before dying or had disintegrated before the next counting event). This may indicate a repellent effect of urine and vernonia. In contrast to the present work, fermented urine is reported to be an attractant for tsetse flies (Okech & Hassanali, 1990). Liquid manure has also been found to change the composition of entomofauna in meadows, but the slight increase of Chrysomelidae under liquid manure compared to the control was not significant (Plewka, 1986). There have been no published studies on the effect of vernonia on insect abundance. Further bioassays are needed to decide on the exact mode of action of urine and vernonia.

The results also show that lambda cyhalothrin, significantly reduced insect numbers in areas adjacent to the treated sub-plots (i.e. the lambda cyhalothrin control subplots). A strong repellent effect could be responsible for the lower insect numbers in the control sub-plots. However, another explanation for these results is that any *O. bennigseni* adults flying onto a lambda cyhalothrin treated plot are killed by the insecticide and these plots act as a "sink" for insects from adjacent areas. This opinion is supported by the fact that dead insects were found in lambda cyhalothrin test sub-plots. There was no significant reduction of *O. bennigseni* adults in control sub-plots for either urine or vernonia, which suggests that the effects are not as strong, or there are two divergent effects.

Bean leaf damage due to adult feeding of O. bennigseni

Insect abundance for the three applications together correlated closely with leaf area loss (r=0.74) for all treatments except lambda cyhalothrin. The regression analysis showed that regression coefficients did not differ significantly for each treatment (Table 2). This leads to the conclusion that there were no significant antifeeding effects of any of the treatments. Other studies report that some *Vernonia* spp. act as an insect feeding deterrent, with the active ingredients being specified as sesquiterpene lactones (Burnett et al, 1974; Rodriguez et al, 1976). The content of sesquiterpene lactones in *V. lasiopus* var. *iodocalyx* still needs to be established, but the present results indicate that it might be one of the species that lacks the feeding deterrent, as no antifeeding effect could be determined. There have been no published studies on the effect of urine on feeding behaviour of phytophagous insects.

TABLE 2: Parameters from correlation and regression analyses of adult *O. bennigseni* abundance per sub-plot of 9 m² on leaf area loss (%). Values are given for all treatments combined and for the four individual treatments, urine, vernonia, lambda cyhalothrin and control, over all three applications.

Treatment	Ν	Correlation	Intercept	Regression	Confidence interval for	
		coefficient		coefficient	regression coefficient	
All	72	0.74	0.80	0.125	0.098 - 0.152	
Lambda cyhalothrin	18	0.23	0.71	0.151	-0.185 – 0.487	
Urine	18	0.85	1.63	0.125	0.084 - 0.166	
Vernonia	18	0.63	0.82	0.147	0.050 - 0.244	
Control (Water)	18	0.89	1.06	0.148	0.108 - 0.188	

Damage to primary leaves, corresponding to early damage after the first application of treatments, was generally low, which was a result of the low numbers of O. bennigseni present on the plants during this time. Urine treated plants had a higher loss of leaf area than the other treatments (Table 3). Although the leaves showed some signs of phytotoxicity by urine, the leaf area loss was certainly caused by *O. bennigseni* as the round holes are very distinct. The middle trifoliate leaves corresponded to the second application of treatments. Leaf area loss in all treatments was still relatively low, in spite of higher insect numbers. Plants in vernonia treated sub-plots had a significantly (P<0.05) lower leaf area loss than the control. Plants in lambda cyhalothrin treated sub-plots had a significantly (P<0.01) lower leaf area loss than control plants. The upper trifoliate leaves correspond to the third application of treatments. The differences between treatments in the leaf damage was less obvious at this time. Only lambda cyhalothrin treated plants showed significantly (P<0.05) less damage than the water treated plants. The correlation between O. bennigseni abundance and leaf area loss demonstrated that reducing abundance leads to less damage, and the different treatments resulted in some differences in leaf area loss, but the damage was generally relatively low.

TABLE 3: Mean percentage leaf area loss for primary leaves (early attack/application 1), middle trifoliate leaves (main attack/application 2) and upper trifoliate leaves (late attack/application 3) after three treatments with water, urine, vernonia or lambda cyhalothrin. Separation of means was done by LSD (P<0.05 and P<0.01). Treatments that are significantly different from the control (water) are shown by * (P<0.05) and ** (P<0.01).</p>

Time	Urine	Vernonia	Lambda	Water	LSD	LSD
			cyhalothrin	(control)	P<0.05	P<0.01
Primary leaves	1.44 *	0.39	0.17	0.39	0.85	1.18
Middle trifoliate leaves	5.83	3.50 *	2.00 **	7.33	3.77	5.21
Upper trifoliate leaves	1.83	2.28	0.94 *	3.06	1.93	2.66

In the study by Karel & Rweyemamu (1984), leaf area losses caused by an abundance of 0.54 *O. bennigseni* per plant were about 40%. In the present study the highest insect abundance was 0.69 *O. bennigseni* per plant (average of insects in one sub-plot for seven counting events after the 2nd application) and resulted in 13% leaf area loss only (average of three leaf area loss calculations). These calculations are dependent on the growth rate of the bean plant during the assessment period. In

the present study the beans grew very fast after the dry spell, and the adult insects inflicted a comparatively lower damage than the insect abundance would suggest.

Larvae abundance of O. bennigseni at harvest and bean yield

Mean larvae numbers in the treatment sub-plots were not significantly different (P>0.05) between treatments (Table 4). The infestation was relatively low compared to a study in the neighbouring district (Hai, Kilimanjaro region) where Ampofo & Massomo, (1998a) estimated an average of 100 larvae/m2. Adult *O. bennigseni* abundance was not correlated to larvae abundance (r=0.12), and larvae abundance did not correlate with the yield (r=0.28). No literature was found on the *O. bennigseni* larvae and bean yield relationship. However, Teixeira et al. (1996) reported that the larvae of *Cerotoma arcuata* Olivier (Coleoptera: Chrysomelidae), which is also root feeding, reduced yield more than the adult feeding on leaves, and their data implied a close correlation between larvae abundance and yield.

Grain yield in test sub-plots is shown in Table 4. In spite of the differences in abundance of *O. bennigseni*, no significant differences in yield between the different treatment sub-plots were found. The trials also showed that decreased adult *O. bennigseni* abundance did not correlate with increased yield (r=0.31). Karel & Rweyemamu (1984) measured a yield gain between 18 and 31% when using synthetic insecticides to control *O. bennigseni* compared to no treatment, but insect abundance was higher than in the present experiment.

	Urine	Vernonia	Lambda cyhalothrin	Water (control)	LSD, P<0.05
Larvae abundance	58.3	33.3	50.0	36.7	36.5
Yield	1840	1710	1840	1830	300

TABLE 4: Mean O. bennigseni larvae abundance per 0.02 m³ soil and mean yield (kg/ha) afterthree treatments with water, urine, vernonia or lambda cyhalothrin.

CONCLUSIONS

The present study demonstrates that vernonia and cow urine are potential natural control substances against *O. bennigseni*. Reduced insect abundance was apparent in both treatments, but duration of the effect was short compared to lambda cyhalothrin. The reduction of *O. bennigseni* abundance led to significantly reduced foliar damage during the peak infestation in beans treated with vernonia.

Hongo & Karel (1986) and Karel (1989) reported similar results with different plant extracts, showing reduced foliar damage after treatment. Insect abundance was only measured once about 24 h after application of the treatments, so the results are inconclusive in regards to the duration of the treatment effect. In the present study, grain yields were generally high and no significant yield gain was achieved by any treatment. Beans are most sensitive to defoliation at the primary leaf stage and during flowering and early pod formation (Gálvez et al, 1977, Cardona et a,. 1982). In the present study the main foliar damage occurred during the later vegetative stages, and no yield decline was measured.

Reduced adult beetle abundance did not directly result in reduced larval abundance in any of the four treatments. It is especially surprising that the standard lambda cyhalothrin did not reduce the larvae population: This means that adult insects must have been able to oviposit in the soil below the treated bean plants prior to dying or without getting in contact with the insecticide. More research is required to distinguish between larvae and adult insect damage, and understand the relationship between adult and larval population.

While the effectiveness of urine and vernonia for the control of *O. bennigseni* have been demonstrated, further research into processes to enhance and prolong the effectiveness of these free and readily available natural substances should be explored, as farmers in Africa need affordable and sustainable methods to become more productive. Pre-harvest infestation of beans (*Phaseolus vulgaris* L.) by *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in relation to bean pod maturity and pod aperture

Submitted to International Pest Management¹

ABSTRACT

Acanthoscelides obtectus is a bruchid beetle that oviposits into ripening bean pods in the field. The larva bores into the seed and develops inside it. The extent of infestation is not easily visible until the first adult generation emerges from the beans. Most farmers have never seen the adult bruchid in their bean field. They therefore do not know that their beans are infested in the field before harvest. However losses due to insects during storage are common and often considered unacceptable by farmers. In this study A. obtectus was trapped for the first time six weeks before harvest in research station fields but only one week before harvest in farmers' fields. For the main harvest in 2003, an infestation rate of 6.9% infested bean seeds was found in dry pods. This is above an economic threshold level of 4% infested seeds. In wilting yellow pods, a lower infestation of 2.5% infested seeds was found. No wilting green pod had been infested. In the minor season 2003/04, after releasing 680 bruchids in a field of 10 bean rows of 10 m length, the infestation rate in dry pods increased to 39% infested seeds, and to 7% in wilting yellow pods, but still no wilting green pods had been infested. This shows that in nature A. obtectus prefers mature dry pods to wilting green pods. In choice laboratorytrials the same applies, and mature pods are more likely to be infested than wilting pods. If the bruchid is not given a choice, it is as likely to infest wilting pods as mature dry pods. Artificially moistening of pods decreased infestation rates. Pod aperture did not consistently influence infestation rates; only in one no-choice laboratory-trial significantly more open pods were infested compared to whole pods. Delayed harvest of mature dry pods in the field with augmented population increased infestation levels from 12% infested seeds to 100% in 14 days. Stimulants for oviposition were evaluated in no-choice trials using different pods, bean seeds, pods without beans and an empty control by counting all eggs laid into the dish during the trial time of six days. Mature dry pods were more stimulating than yellow wilting and green wilting pods. Moistened or not moistened pods did not differ in their stimulation, nor did open or closed pods. Bean pods containing seeds were as stimulating as bean seeds alone, and significantly more stimulating than empty opened bean pods where seeds have been removed. Bean pods without seeds were still more stimulating than the empty control. Farmers are advised to harvest as early as possible and dry their beans in a place without exposing them to A. obtectus adults. Future research should concentrate on proving where pre-harvest infestation originates from.

¹ Paul UV, Hilbeck A, Edwards PJ, submitted 2008, International Pest Management

INTRODUCTION

Stored beans suffer heavy losses in terms of both quality and quantity (Abate & Ampofo, 1996). Farmers in East Africa suggest total bean loss to occur after three to four months in storage if nothing is done to prevent the infestation. These losses are mostly caused by insects, of which Acanthoscelides obtectus (Say) and Zabrotes subfasciatus (Boheman) are the most important ones in Africa (Giga et al. 1992). They occur together and have a similar biology, but differ in ways of infestation: Z. subfasciatus infests threshed beans, but not whole bean pods. The adult female glues its eggs onto the bean seed, and the larva bores straight into the seed (Abate & Ampofo, 1996). In contrast A. obtectus may infest growing pods by chewing an opening into the suture and laying an egg cluster into the pod cavity (Zachariae, 1958). Hatched larvae of *A. obtectus* wander among the beans before penetrating them (Parsons & Credland, 2003). The newly emerged adult A. obtectus mates within the first 24 hours after emergence and normally starts ovipositing during the next 24 hours (Parsons & Credland, 2003). The adult may feed on sugar water, but does not normally feed. It ingests pollen, but this seems to be mainly due to licking bean leave surfaces (Jarry, 1987).

In Moldavia, *A. obtectus* were seen on bean plants about one month before they were observed ovipositing on beans during late pod formation. The insect was therefore present on beans almost two months before harvest (Sapunaru et al, 2006). In Zimbabwe *A. obtectus* occurred in small numbers in bean fields about two months before harvest, and numbers increased about one week before harvest (Giga & Chinwada, 1993). *A. obtectus* was found on various plants as much as on bean plants during summer in Germany (Zachariae, 1958). Zachariae suspects they came from infested seeds that were planted in spring. Labeyrie (1962) counters this as not probable, because females would not oviposit after a few weeks of being an adult. He suggests that the infestation in the field results from adults emerging from stored beans. He reports that they were attracted by light, and if the temperature was above 20°C, they migrated out of the store and travelled up to 3 km.

A. obtectus females are stimulated to oviposit by the presence of beans (Huignard, 1976, Monge, 1983). It is probable that in the field the wilting pods attract the females (Zachariae, 1958). He observes more females on soft wilting and on wet mature pods than on dry mature ones. He concludes that the wet, wilting or mature bean pod

emits an attractant odour. Jarry & Chacon (1983) report higher field infestation rates by *A. obtectus* in partially opened pods compared to closed pods. They assume higher attraction to those pods. Female *A. obtectus* seem to use pod openings for ovipositing, but some dehiscent pods also present orifices made by the insect. Higher contamination rates with partially opened pods (here mainly holes made by *Helicoverpa* spp.) were also found by CIAT (2001) in Tanzania.

Authors don't agree on the period of susceptibility. Some suggest that infestation only occurs during two weeks before harvest (Schmale et al 2002), or at maturity (Labeyrie, 1962). Others report that *A. obtectus* oviposits into green and hardly wilted pods (Zachariae, 1958), or at a reduced rate, even during pod formation (Menten & Menten, 1984, Labeyrie & Maison, 1954).

Due to the nature of storage, economic thresholds are difficult to establish (Haines, 2000). This is even more so in beans, where the pungent smell of infested beans and the clearly visible emergence holes make them unmarketable before a significant weight loss occurs. The acceptable threshold of infestation is considerably lower than in cereals (Giga et al, 1992). The only quantified threshold is 4% of infested seeds mentioned by Baier & Webster (1992). There is no threshold estimation on acceptable levels for field infestation. Measured infestation rates vary greatly for locations and years. Table 1 summarises infestation rates from literature.

Author	Country	Infestation/contamination ¹	Variables
Jarry et al, 1987	France	26-95% infested pods	Date of maturity
Jarry & Chacon, 1983	France	40-68% infested pods	Time of exposure
Paul, unpublished	Tanzania	0.0-9.3% infested pods	Maturity stage
Giga & Chinwada, 1993	Zimbabwe	~10% infested pods	Storage duration
Sapunaru et al, 2006	Moldovia	0.01-3.0% infested seeds	Location, year
Schmale et al, 2002	Colombia	0.0-5.5 adults/100 seed	Location, year
CIAT, 2001	Tanzania	~1.5-16 adults/100 seeds	Time of exposure, aperture
Labeyrie, 1962	France	1.3-7 adults/100 seeds	Companion crop
Labeyrie, 1957	France	6.4-30.3 adults/100 seeds	Time of exposure
Menten & Menten, 1984	Brazil	7.5-169.25 adults/100 seeds	Maturity stage (with augmented population)

TABLE 1: Summary of field infestation and contamination rates as reported by different authors.

¹ Throughout this text, we use 'infestation' relating to number of pods or seeds containing one or more egg/larva/adult and 'contamination' relating to the number of eggs/ larvae/adults per pod or seed respectively.

In Europe, early maturing beans are more infested than late maturing ones (Jarry et al, 1987, Zachariae, 1958). Labeyrie (1962) contradicts this saying that early planting

of fast maturing beans should reduce infestation. This probably depends on prevalent temperatures, as the reproductive activity of *A. obtectus* is much reduced below 20°C (Labeyrie, 1962). It is generally agreed that leaving the crop in the field after maturity prolongs the time of exposure to the insect and increases infestation (CIAT, 2001, Olubayo & Port, 1997, Jarry & Chacon, 1983). The same applies to delayed threshing (Labeyrie, 1957), because this gives the larvae the chance to penetrate the bean seed before they might be shaken off or crushed during threshing. In regard to recommendations Lima (1987) suggests harvesting at physiological maturity with subsequent fast drying. Contrary Olubayo & Port (1997) conclude that the farmer will not reduce bruchid infestation in cowpeas (also partly caused by *A. obtectus*) by harvesting earlier than recommended, which is at a seed moisture content of about 14%.

Most farmers in East Africa do not realize that beans are infested before harvest (Paul, unpublished, Giga et al, 1992). Nor do they understand the beetle's lifecycle, or the origin of the damage exclusively done by the larvae. Yet they know from experience that late harvesting increases the problem of bruchid infestation. Even so, farmers allow their crops to mature in the field to storage dryness, and only harvest beans early and dry them at the homestead where wetter conditions prevail (Giga et al, 1992). Threshing is normally done in the first week after harvest with exceptions notably in areas where A. obtectus is not prevalent (Giga et al, 1992). The beans may be sorted and sunned before using one of several storage practices. Industrial insecticides keep losses at a minimum, but many farmers do not use them because of high costs and poor accessibility, but also because of fake chemicals on the market and farmers' incapability to authenticate the product (Paul, Lossini, unpublished). Traditional control measures often incur a 50% loss, and therefore farmers often sell the bulk of their crop soon after harvest (Giga et al, 1992). Elimination of field infestation, together with other low cost preventive methods, could encourage farmers to store their crop until prices have normalised after harvest and thereby increase their profit considerably.

In order to determine the most probable infestation time, this study investigates the occurrence of *A. obtectus* in maturing bean fields in Northern Tanzania, and studies infestation rates in the farmers' fields in relation to pod maturity. Because the natural infestation rate was very low, the trial was repeated in a field on the research station with an artificially augmented *A. obtectus* population. Other examined factors were

pod aperture (natural and artificial) and the distance between fields and homesteads, where the most probable source of infesting bruchids lies. The results of the field infestation rates were compared with a series of choice and no-choice laboratory assays to study the insect's preferences. These laboratory assays explore in addition stimulators of oviposition in *A. obtectus* females, such as dry pods compared to wet pods, pods (without seeds) or bean seeds alone. The results are discussed in the light of possible actions to reduce pre-harvest infestation by small scale farmers in Northern Tanzania.

MATERIALS AND METHODS

Part 1: Occurrence of adult A. obtectus in maturing bean fields

Part 1 studies the presence of adult *A. obtectus* in ripening bean fields, to establish a first possible infestation of beans in the field.

Nine bean fields of approximately 1000 m² each were selected during the long rainy season (Masika) of 2003 for their location to potential sources of infested beans. They were planted between the end of March and the end of April.

Six fields were research fields, located at the Selian Agricultural Research Institute (SARI; 3°22'S, 36°37'W, elevation 1387 masl, mean temperature 21°C, see number 1 in Fig. 1), two of these fields were adjacent to each other, and were located about 1 km from research buildings where beans are stored. One of these fields was planted with multiple climbing bean varieties, and the other field was planted with the Calima type bean cultivar Lyamungo 90. The four other fields at the research station were close to the research building and residential houses, and were planted with multiple bean varieties from the CIAT (Centro Internacional de Agricultura Tropical) core collection.

The three farmers' fields were located at Tengeru (Patandi village, Duluti subvillage: 3° 24 S, $36^{\circ}47$ E, elevation 1205 masl, mean temperature 22° C; see number 2 in Fig.1)). One of these fields was about 1 km from any housing or storage building and the two other fields were each adjacent to residential houses. They were planted with the bean cultivar Lyamungo 90. From mid May, four traps per field were randomly placed into each 1000 m² bean field before physiological maturity (CIAT, 1986b). The transparent polythene sheet of each trap measured 20 x 40 cm. It was mounted to a frame made with 4 mm wire of 80 cm height. Sticky glue was applied to both sides of the sheet. The trap was stuck into the soil with the longer sides of the sheet parallel

to the ground and the shorter sides perpendicular to the ground. The lower side of the polythene sheet was situated about 5 cm above the bean canopy. Each sheet was facing a different direction: N-S, E-W, NW-SE, and NE-SW. The polythene sheet was changed and trapped bruchids were counted and identified each week for about eight weeks. The traps were removed in the week after harvest.



FIGURE 1: Sketch of sampling locations for all trials (scale: Arusha – Moshi: approx. 80 km).

- 1: Selian Agricultural Research Institute (3º22'S, 36º37'W, 1387 masl)
- 2: Tengeru (3º 24 S, 36º47 E, 1205 masl)
- 3: Ol'kungwado (3º 07 S, 36º51 E, 1410 masl)
- 4: Kikatiti (3º 23 S, 36º57 E, 1090 masl)
- 5: Fuka (3º 12 S, 37º06 E, 1230 masl)

Part 2: Natural infestation by A. obtectus in farmers' bean fields

Part 2 assesses the infestation of bean pods by *A. obtectus* in farmers' fields, by harvesting beans of various maturities and incubating them individually until the emergence of *A. obtectus* adults.

Between 4 and 15 July 2003, one to four days before harvest, a total of 2915 bean pods were collected from eight different farmers' bean fields, two of these fields were fields monitored in part 1 (Tengeru, both close to homesteads, see number 2 in Fig. 1). In each of the other three villages (see numbers 3, 4 and 5 in Fig. 1), two fields were selected based on anticipated harvest date. The cultivar of beans and distance to the closest house was noted. In two transects on the same day, 50 bean plants were randomly collected. After returning to the research station, the pods were classified into (1) three maturity classes: physiological maturity to wilting stage (R8B,

still green), wilting stage (R9A, discoloured, yellow) and harvest maturity (R9B, dry, light brown) (CIAT, 1986b), and (2) three different pod characteristic classes (whole pods, pods with holes (e.g. made by *Helicoverpa* spp.) and dehiscent pods giving bruchids an easy access into the pod). Pods were visually checked for oviposition holes by A. obtectus (Zachariae, 1958). Pods were carefully opened (not to disturb hatching) and eggs and/or larvae were counted. Preliminary tests showed that this procedure does not influence hatching success (Paul, unpublished). Each opened but complete pod (with seeds, eggs/larvae) was placed individually in a plastic petridish of 9 cm diameter. The petridish was closed and kept for 48 hours at 30± 1°C and a humidity of 40±5% r.h. According to Labeyrie (1962), these are the best conditions for high survival of eggs. Afterwards the dishes were kept at ambient temperature of 19±2°C, 60±10% r.h. and a photoperiod of 12 hours. The petridishes were checked weekly for emerging adult A. obtectus and emergence holes per bean were counted. Emerging adults mate in the first 24 hours and start oviposition in the following 24 hours (Parsons & Credland). A continued infestation could therefore not be excluded and consequently counting continued only until one month after the first emergence of adult beetles to exclude the counting of adults from the second generation.

Part 3: Infestation by A. obtectus in bean fields with augmented population

To validate the data on infestation preferences of *A. obtectus* in the field, a trial with released *A. obtectus*, and therefore higher infestation levels, was designed for the short rainy season (Vuli) 2003/04. *A. obtectus* were collected from farmers' beans and reared for several generations on threshed dried beans as described by CIAT (1986a) at $21\pm3^{\circ}$ C, $60\pm10\%$ r.h. and with a 12 hour photoperiod. On 1 December 2003 at SARI (number 1 in Fig. 1), ten rows of 10 m of beans were planted with the recommended practice of two beans per planting hole, 20 cm apart within the row and 50 cm between rows. The plot was weeded once on 14 December. On 11 and 12 February 2004, one to two day old *A. obtectus* adults (360 and 320 respectively) were released at regular distances between the rows of beans. In regular two – three day intervals until 27 February, four plants per row were harvested and all pods were classified into the same three maturity classes and the same three pod characteristic classes as in part 2 (see above). In total 1473 pods were carefully opened (not to disturb hatching) and eggs were counted. Each pod (with seeds and eggs/larvae)
was then placed in a plastic petridish of 9 cm diameter. The petridish was closed and kept at ambient temperature of $21\pm2^{\circ}$ C, $60\pm10\%$ r.h. and a 12 hour photoperiod. The petridishes were checked twice weekly for emerging adult *A. obtectus* and emergence holes per bean were counted until one month after the first emergence of adult beetles as explained above.

Part 4: Infestation preferences of *A. obtectus* studied in choice and no-choice laboratory assays

Additional laboratory trials with controlled conditions were designed to complement the data from the field trials. These assays explored oviposition stimulation and preferences of A. obtectus females in more detail. At SARI (number 1 in Fig. 1), beans were raised continuously in a screen house to produce non-infested pods at different maturity stages for the laboratory trials during the short rainy season (Vuli) 2003/04. Pods with no blemish (i.e. no dehiscent pods, no pods with hole or any signs of disease) at the required maturity stage were harvested on the day starting each assay. The pods were treated as described for the different assays below. All assays were conducted at ambient temperature of 21±2° C, 60±10% r.h. and a 12 hour photoperiod. In all assays six unsexed one to two day old A. obtectus (see trial 3 above) were added to each petridish of 9 cm diameter. The petridish was closed and after five days the six beetles were removed, killed and sexed. All petridishes contained females and males, and as shown in another study, the female/male relation does not influence total oviposition in confined spaces (Paul, submitted, unpublished). Because of this total numbers per pod are presented here. All pods were checked for oviposition holes, some pods were carefully opened (not to disturb hatching), and eggs were counted in two locations: (1) in the bean pod (i.e. successful oviposition into the pod), and (2) in the petridish beside the pod (i.e. oviposition stimulated, but no successful penetration of the pod). Each pod with its eggs (but not the eggs in the petridish) was kept in individual petridishes. From four weeks onwards (the earliest possible date of emergence), the petridishes were checked twice weekly for emerging A. obtectus adults. Numbers of beetles and emergence holes per beans were counted until one month after the first emergence of adult beetles (as explained above). The individual procedures for each assay are explained in detail in the next four paragraphs.

a) Choice assay: Preference based on maturity stages of bean pods

Two pods of different maturity (same classes as in part 2) were placed into one petridish. Each combination of the three maturity stages was repeated sixteen or seventeen times. All pods were opened after the adults have been removed, and eggs in the petridish as well as in the two pods were counted. The two pods were then kept in individual petridishes.

b) No-choice assay 1: Maturity stage and pod aperture

A total of 138 pods of different maturity as above were collected and 50% of the pods were split manually before adding the adult bruchids. After removal of the adults, about half the closed pods were opened and eggs in all open pods were counted (about 75% of all pods). The data were used to assess oviposition and to ascertain that pod opening did not disturb the development of *A. obtectus*.

c) No-choice assay 2: Mature, dry and artificially soaked pods

A total of 77 mature dry pods were collected and 50% of the pods were soaked for five minutes in a dish with clean water. After removal of the adults, about half the pods were opened and eggs were counted (see above under b).

d) No-choice assay 3: Mature pods or dry beans

Seventy two mature dry pods were each put separately into a petridish, and six one to two day old unsexed *A. obtectus* adults were placed together with each pod. Forty other dry pods were opened, the seeds removed and the pod (without the seeds) were put into individual petridishes. Then they were treated as the whole pods above. Forty more petridishes were filled with 9-12 dry non-infested beans of the same age, and then treated like the petridishes with pods. As a zero control thirty-four petridishes were left empty, but received six adult beetles, and were treated as the others. This experiment explores oviposition stimulants. After removal of the adults, all pods were opened and eggs were counted in all treatments.

Statistical analysis

a) Part 1: The data is not normally distributed. A square-root-transformation was applied before analysis. ANOVA for the total sum of trapped beetles in each trap was calculated for the factors "distance to houses" with the expressions "close" (less than 1 km to nearest house) and "far" (1 km or more to nearest house); and "field type" with the expressions "farmers' fields" and "research fields". ANOVA and LSD for separation of means were used for analysing the time effect ("weeks before harvest") on trapped beetles.

b) Part 2 to 4: All trials were to some degree unbalanced due to harvest sampling (part 2 and 3) and availability of young adult *A. obtectus* (part 4). The data were not normally distributed, and did not fulfil the requirement of homoscedasticity. Transformations were not able to remedy this situation. Therefore Duncan's C test was used for part 2 and 3 (big samples), and Duncan's T2 test for separation of means at p<0.05 for part 4 (small samples) (Anonymous, not dated). The two tailed t-test for paired samples (p<0.05) was used for analysing the choice assay in part 4. All statistical procedures were calculated using SPSS 14.0 and MS Excel 07.

In Table 2 all parts of this study are summarised with indications on location, number of fields and pods sampled, used bean cultivars, time of trial, main objectives and indication of used methods.

Part	Loca-	No.	Bean	Year and	Total	Main objective	Method used
No.	tions	fields	cultivars	season	pods	regarding A. obtectus	
1	1,2	9	Diverse,	Masika 03	n.a	Period of occurrence in	Sticky traps in fields from
			incl. Lya			farmers' fields	physiological maturity to harvest
2	2,3,4,5	8	Lya, Nka	Masika 03	2915	Natural infestation rates	Collection in farmers' fields: pod
						in farmers' fields	maturity, aperture, distance
3	1	1	Lya	Vuli 03/04	1473	Infestation preferences	Augmented population:
						in natural infestation	pod maturity, aperture
4a	1	lab.	Lya	Vuli 03/04	98	Oviposition preferences	Choice tests: maturity of pod
4b	1	lab.	Lya	Vuli 03/04	138	Oviposition preferences	No-choice: maturity, aperture
4c	1	lab.	Lya	Vuli 03/04	77	Oviposition preferences	No-choice: moistened pod
4d	1	lab.	Lya	Vuli 03/04	186	Oviposition stimulation	No-choice: pods or beans

TABLE 2: Summary of all trial parts: please refer to the text for description of treatments and factors

Remarks: Location: 1: SARI, 2: Tengeru, 3: Ol'kungwado, 4: Kikatiti, 5: Fuka (see Fig. 1)

Bean cultivars: Diverse: climbing and bush types, Lya: Lyamungo '90 (improved), Nka: Nkamna (traditional) Year and season: Masika: March – June (harvest: July), Vuli: November – January (harvest: February) Total pods: all treatments (including treatments with beans and no pods in trial 4d)

RESULTS

Part 1: Occurence of adult A. obtectus in maturing bean fields

In total 19 *A. obtectus* were trapped in 14 of 36 traps. Seventeen *A. obtectus* (mean \pm SE: 0.71 \pm 0.15 *A. obtectus*/trap in eight weeks) were trapped in fields close to houses and only two *A. obtectus* (mean \pm SE: 0.17 \pm 0.21 *A. obtectus*/trap in eight weeks) were trapped in a field more than one kilometre from any houses in a dry, mature crop, one week before harvest. This means that significantly less (p<0.05) *A. obtectus* were trapped in far fields than in close fields.

No *A. obtectus* was trapped during week seven before harvest. Two *A. obtectus* (mean \pm SE: 0.056 \pm 0.049 *A. obtectus*/trap in one week) were trapped six weeks before harvest in a physiological mature crop (end of stage R8A (CIAT, 1986b), water content of seed about 60%). This field was situated close to a field that was ready for harvest. Two *A. obtectus* (mean \pm SE: 0.056 \pm 0.049 *A. obtectus*/trap in one week) were trapped weekly between five to two weeks before harvest in fields with wilting bean pods (stage R9A, water content of seed about 30%). Nine *A. obtectus* (mean \pm SE: 0.25 \pm 0.05 *A. obtectus*/trap in one week) were trapped one week before harvest in fields with beans dry and mature for harvest (stage R9B, water content of bean seed about 15%). No *A. obtectus* was trapped after harvest. This means that significantly more *A. obtectus* were trapped in the week before harvest than in any other week of the study (p<0.001 and LSD (p<0.05)=0.134).

Of the 19 *A. obtectus*, six (mean \pm SE: 0.50 \pm 0.23 *A. obtectus*/trap in eight week) were trapped in farmers' fields, close to houses. Thirteen *A. obtectus* were trapped on research fields (mean \pm SE: 0.54 \pm 0.16 *A. obtectus*/trap in eight weeks). There was no significant difference in presence of *A. obtectus* in farmers' and research fields, but there was a significant interaction (p<0.05) between the field type (farmers fields and research fields) and time (weeks before harvest). *A. obtectus* was present in research fields from week six until week one before harvest (two to three per week), in contrast in farmers' fields, all six *A. obtectus* were found in the week before harvest (Fig 2).



FIGURE 2: Mean numbers (±SE) of trapped *A. obtectus* per trap during different weeks before harvest in fields at the research station (SARI) or farmers' fields in Tengeru.

Part 2: Natural infestation by *A. obtectus* in farmers' bean fields

Significantly more adult *A. obtectus* per 100 seeds emerged from fields close to homesteads than from fields more than 1 km away from any houses ($p \le 0.001$). There were also significantly more infested pods and infested beans in "close fields" than in "far fields" (p < 0.001). No *A. obtectus* emerged from any seed that was between physiological maturity and "wilting green" stage (R8B stage). This was significantly less than in the other two maturity classes (p < 0.001). The classes "wilting yellow" (R9A) and "dry" (R9B) were not significantly different at p < 0.05. More mature pods had significantly more infested pods and beans than less mature pods (p < 0.001) for all three maturity classes. There were no significant differences between any of the pod opening characteristics at p < 0.05. See Table 3 for the detailed results.

Part 3: Infestation by *A. obtectus* in a bean field with augmented population

No *A. obtectus* emerged from any seed that was between physiological maturity and "wilting green" stage (R8B). This was significantly less than in the other two maturity classes (R9A and B), which were also significantly different to each other (p<0.001). More mature pods had significantly more infested pods and beans than less mature pods (p<0.001). There were no significant differences between any of the pod opening characteristics at p<0.05 (Table 3).

Later harvest dates resulted in heavier infestation, as expressed in emerged adult *A. obtectus* per 100 seeds, or percent infested pods, or infested beans. This was independent whether one looked at a complete harvest and did not discriminate between the different maturity stages (as it would normally be when a farmer harvests his beans) or if one looked only at the most mature pods (R9B) for each date (Table 2). When considering the "wilting yellow" maturity class (R9A) only, infestation and contamination rates did not differ significantly for different harvest dates and ranged between 0.08 and 0.77 *A. obtectus*/100 seeds, 1.6% and 22.6% infested pods, and 1.6% and 35.5% infested bean seeds (Table 3).

Part 4: Infestation preferences of *A. obtectus* studied in laboratory choice and no-choice assays

a) Choice assay: Preference based on maturity stages of bean pods

In the laboratory trials all maturity classes were infested and *A. obtectus* adults emerged from pods of all classes. When given the choice, *A. obtectus* preferred the relatively more mature pods (R9A and B) to the "wilting green" pods (R8B), confirming the field results. This preference was less distinct between the "wilting

green" and "wilting yellow" pods, but still led to statistically significant differences for % of infested pods. There was no significant difference in any result for the pairs "wilting yellow" and "dry" pods (Table 4).

TABLE 3: Means (±SE) of emerging adult *A. obtectus* per 100 seeds, % infested pods and % damaged beans at different harvest dates. Different letters behind the data indicate significant differences (p<0.05.) between the means in the same column and part as determined by Duncan's T2 test. Maturity stages: R8B: between physiological maturity and start of wilting stage (pods are still green), R9A: wilting stage (yellow pods) and R9B: dry harvest maturity.</p>

			A. obtectus/100 seeds		% infeste	d pods		% infeste	d beans o	or
	N=	mean	±SE		mean	±SE		mean	±SE	
Part 2										
close	1748	4.0	± 0.7	а	2.9	± 0.3	а	4.6	± 0.5	а
far	1167	0.3	± 0.8	b	0.3	± 0.4	b	0.5	± 0.7	b
R8B stage	662	0.0	± 0.0	а	0.0	± 0.0	а	0.0	± 0.0	а
R9A stage	1574	2.4	± 0.7	b	1.4	± 0.3	b	2.5	± 0.6	b
R9B stage	679	5.3	± 1.1	b	4.6	± 0.5	С	6.9	± 0.9	С
Closed	1500	1.8	± 0.7	а	1.8	± 0.3	а	2.6	± 0.6	а
Holes	1225	2.7	± 0.8	а	1.8	± 0.4	а	3.3	± 0.7	а
Split	190	6.7	± 2.0	а	2.1	± 1.0	а	3.7	± 1.8	а
Part 3										
R8B stage	109	0.0	± 0.0	а	0.0	± 0.0	а	0.0	± 0.0	а
R9A stage	296	5.5	± 6.0	b	4.7	± 2.1	b	7.1	± 4.3	b
R9B stage	1068	43.3	± 9.2	С	21.7	± 1.1	С	39.0	± 2.3	С
Closed	1225	31.5	± 3.0	а	16.5	± 1.1	а	29.3	± 2.2	а
Open	248	37.4	± 6.6	а	17.7	± 2.4	а	31.5	± 4.8	а
All maturity c	lasses									
13 February	283	6.1	± 6.0	а	3.2	± 2.1	а	4.9	± 4.3	а
16 February	259	15.1	± 6.3	а	10.8	± 2.2	b	16.6	± 4.5	а
18 February	255	14.3	± 6.3	а	10.2	± 2.3	b	14.5	± 4.6	ab
20 February	240	47.1	± 6.5	b	24.2	± 2.3	с	43.8	± 4.7	с
23 February	222	45.8	± 6.8	b	26.6	± 2.4	с	45.9	± 4.9	с
25 February	198	72.5	± 7.2	b	29.8	± 2.6	С	60.1	± 5.2	С
27 February	16	74.9	±25.3	ab	43.8	± 9.0	abc	100.0	±18.2	abc
Only dry class	s (R9B)									
13 February	101	13.0	±11.6	а	6.9	± 4.0	а	11.8	± 8.2	а
16 February	165	21.6	± 9.1	ab	15.8	± 3.1	ab	23.6	± 6.5	а
18 February	198	17.9	± 8.3	а	12.6	± 2.9	а	18.2	± 5.9	ab
20 February	197	54.2	± 8.3	С	25.9	± 2.9	bc	47.7	± 5.9	С
23 February	203	49.7	± 8.2	bc	28.6	± 2.8	с	49.8	± 5.8	С
25 February	187	76.4	± 8.5	С	31.0	± 3.0	с	62.6	± 6.1	С
27 February	16	74.9	±29.2	abc	43.8	±10.1	abc	100.0	±20.8	abc

TABLE 4: Means of eggs found inside pods, numbers of emerging *A. obtectus* per pod, and percent infested pods in pairs of pods of different maturity. The p value behind each data pair indicates the statistical significance of the two means being different as determined by the pairwise t-test (error probability). Maturity stages: R8B: between physiological maturity and start of wilting stage (pods are still green), R9A: wilting stage (yellow pods) and R9B: dry harvest maturity.

	R8B (1) -	R9A (2) po	ds	R8B (1) -	- R9B (2) po	ds	R9A (1) – R9B (2) pods		
	Mean 1	Mean 2	p=	Mean 1	Mean 2	p=	Mean 1	Mean 2	p=
Eggs in pod	10.06	12.62	0.66	2.41	19.35	0.00	20.25	18.00	0.61
A. obtectus/pod	4.25	11.25	0.14	3.76	17.59	0.01	8.31	8.50	0.96
% infested pods	31.25	75.00	0.01	41.18	76.47	0.03	75.00	56.25	0.33

b) No-choice assay 1: Maturity and pod opening

When no choice was given, *A. obtectus* oviposited similar numbers of eggs into pods of all maturity stages. The emerging adult *A. obtectus* per pod or proportion of infested pods were not significantly different. More eggs however were found in the petridishes with "dry" pods (R9B) than with "wilting green" pods (R8B) (Table 5).

TABLE 5: Means (±SE) of eggs found inside pods, in the dish beside pods, numbers of emerging *A. obtectus* per pod, and % infested pods in wilted (R8B), yellow (R9A) or dry (R9B) pods. Different letters behind the data indicate significant differences (p<0.05.) between the means in the same column as determined by Duncan's T2 test.</p>

	Eggs in	pods p	=0.29	92)		Eggs in dish (p=0.025)			
	mean	±SE		n=		mean	±SE		n=
R8B stage	9.5	±6.5	а	10	-	4.3	±2.9	а	20
R9A stage	21.6	±6.2	а	11		10.8	±3.0	ab	18
R9B stage	20.8	±3.8	а	30		12.9	±1.3	b	100
	A. obte	c <i>tus</i> /po	d (p=	0.381)		% infest	ted pod	s (p=0.1	62)
	moon	TCE		n-		moon	+SE		n-

	mean	±5E		n=	mean	±SE		n=
R8B stage	9.0	±2.1	а	20	55.0	±10.7	а	20
R9A stage	9.9	±2.3	а	18	83.3	±11.3	а	18
R9B stage	6.9	±1.0	а	100	63.0	± 4.8	а	100

Opened pods were preferred to closed pods for oviposition as shown in the infestation rate (% infested pods). Surprisingly however, no significant differences in the contamination rates were found (expressed in number eggs inside pods/pod, number eggs in the dish/dish (pod), or emerged *A. obtectus*/pod; Table 6).

c) No-choice assays 2: Mature, dry and artificially soaked pods

Significantly more eggs were laid into dry than into soaked pods, and more dry pods were infested than soaked pods. No significant differences in emerging insect numbers or in eggs laid into the dish were found (Table 7).

TABLE 6: Means (±SE) of eggs found inside pods, in the dish beside pods, numbers of emerging *A. obtectus* per pod, and % infested pods in closed or artificially opened (split) pods. Different letters behind the data indicate significant differences (p<0.05.) between the means in the same column as determined by Duncan's T2 test.

	Eggs in	pods				Eggs in			
	(p=0.210))				(p=0.123			
	mean	±SE		n=		mean	±SE		n=
Closed pods	22.8	±4.3	а	23	_	9.6	±1.6	а	67
Open pods	15.4	±3.9	а	28		13.1	±1.6	а	71
	A. obteo	ctus/po	d			% infested pods			
	(p=0.802	2)				(p=0.02)	7		
	mean	±SE		n=		mean	±SE		n=
Closed pods	7.8	±1.2	а	67	_	55.2	±5.8	а	67
Open pods	7.4	±1.1	а	71		73.2	±5.6	b	71

TABLE 7: Means (±SE) of eggs found inside pods, in the dish beside pods, numbers of emerging *A. obtectus* per pod, and % infested pods in mature dry pods that were either left dry or soaked by immersing for five minutes in water. Different letters behind the data indicate significant differences (p<0.05.) between the means in the same column as determined by Duncan's T2 test.

	Eggs in	pods (p	o=0.0	47)		Eggs in dish (p=0.133)					
	mean	±SE		n=		mean	±SE		n=		
Soaked pods	22.9	±5.7	а	19	-	8.7	±2.5	а	38		
Dry pods	39.5	±5.7	b	19		14.1	±2.5	а	39		
	A. obtec	ctus/po	d (p=0	0.777)		% infested pods (p=0.027)					
	mean	±SE		n=		mean	±SE		n=		
Soaked pods	9.6	±1.9	а	38	-	52.6	±7.5	а	67	_	
Dry pods	10.4	±1.9	а	39		79.5	±7.4	b	39		

d) No-choice assays 3: Mature pods or dry beans

A. obtectus laid more eggs into dishes with closed complete pods (with bean seeds) than dishes with pods where the bean seeds were removed. Pods alone (without bean seeds) stimulated *A. obtectus* to lay more eggs than empty dishes. Closed pods with bean seeds were of similar preference than bean seeds without pods in

regard to number of eggs laid, number of emerged *A. obtectus*, and proportion of infested dishes (Table 8)

TABLE 8: Means (±SE) of total eggs found in the dish (including inside pods), numbers of emerging *A. obtectus* per dish, and % dishes with at least one emerging adult in dry unopened pods, pods without beans (removed beans), dry beans alone and empty dishes. Different letters behind the data indicate significant differences (p<0.05.) between the means in the same column as determined by Duncan's T2 test.</p>

	Number	eggs			Number	A. obt	ectus	/ pod	% infested pods/dishes			
	(p<0.001	(p<0.001)			(p<0.001)				(p<0.001)			
	mean	±SE		n=	mean	±SE		n=	mean	±SE		n=
Pods (+ bean seeds)	29.0	±3.5	С	47	3.5	±1.0	b	72	36.1	±4.5	b	72
Pods (- bean seeds)	12.1	±3.8	b	40	0.0	±0.0	а	40	0.0	±0.0	а	40
Bean seeds (- pod)	26.3	±3.8	bc	40	9.4	±1.3	b	40	62.5	±6.0	b	40
Control empty petridishes	5.2	±4.1	а	34	0.0	±0.0	а	34	0.0	±0.0	а	34
(- bean seeds, - pod)												

DISCUSSION

In essence, when given a choice, *A. obtectus* prefers mature bean pods (stage R9A or R9B), and has higher rates of oviposition and infestation in the more mature and dry pods. However, as shown in the laboratory trials, *A. obtectus* is fully capable to survive on less mature pods (e.g. R8B stage), and produces similar numbers of offspring in no-choice trials with less mature bean pods than with mature pods. The disagreement in literature on the stage of susceptibility can therefore be explained: field studies necessarily give *A. obtectus* a choice, and therefore find infestation in mainly mature dry pods (e.g. Schmale et al., 2002), and laboratory studies with a limited or no choice component find reproduction to take place in a wider range of maturity stages (e.g. Menten & Menten, 1984).

This study indicates that both pod and bean seeds contribute jointly to the oviposition stimulation of the adult female *A. obtectus*, measured by numbers of eggs oviposited into each dish. This conclusion was reached since pods without bean seeds stimulated oviposition more than the control without bean seeds or pods, and bean seeds alone or complete pods (including bean seeds) stimulated oviposition more than empty pods alone. Stimulation did not differ significantly between open pods (with exposed seeds) and closed pods (with enclosed seeds), which supports this thesis further. No difference in total oviposition stimulation between dry and wet pods was found in this study. This is contrary to Zachariae's (1958) observation that

more females were found on mature pods after rain than during dry spells. He concluded that wet pods would emit more attractant volatiles compared to dry pods. Although not directly comparable because Zachariae did not measure oviposition, this study does not support his findings. However, oviposition stimulation was significantly increased by more mature pods compared to less mature pods at stage R8B (physiological maturity until early wilting stage), but not between the two more mature stages (R9A and R9B). Zachariae (1958) reported more oviposition into moist mature (probably stage R9A) than dry mature pods (R9B), which can not be confirmed. In conclusion, oviposition stimulation is probably not regulated by the moisture in the pod wall, but by another stimulant related to the maturity of the pod and/or the bean seed which still has to be described. This study would also need to study tactile stimuli in oviposition behaviour, since wet pods of the same age are less preferred than dry pods.

For the first time it is shown that less adult *A. obtectus* were trapped in fields located more than 1 km away from houses than in fields closer to homesteads, and that infestation rates were significantly lower in beans from "far" fields than from close fields. This supports the hypothesis by Labeyrie (1962) that bruchids escaping from infested bean stock are responsible for field infestation, and therefore far fields are less likely to be infested. The closest study is by Schmale et al. (2002) who found that bean fields away from main bean producing areas were less infested than fields in major bean producing areas. This mainly proves that higher pest pressure increases infestation, but does not prove different levels of infestation in fields, dependant on distance to possible infestation sources. Farmers should be advised to plant beans whenever possible on fields away from storage places to reduce preharvest infestation. More research is needed to study dispersion of *A. obtectus*.

In this study *A. obtectus* adults were not trapped in farmers' fields until one week before harvest, when it could find the preferred mature dry pods. In contrast, other studies found *A. obtectus* to be present in bean fields about two months before harvest (Sapanuru, 2006, Giga & Chinwada, 1993, Zachariae, 1958). This late occurrence of adults in the maturing field explains why under natural infestation no wilting pod has been infested. On the research station however, adult bruchids were found six weeks before harvest in fields without mature pods, where neighbouring fields were close to harvest. Theoretically, infestation could take place in such circumstances, but is unlikely, due to the preference of the *A. obtectus* for more

mature bean pods in neighbouring fields. Further studies are needed to decide if planting a field with early beans could act as a trap crop for *A. obtectus* and reduce infestation in a later main crop, or whether this practice would increase the presence of bruchids and result in higher infestation in the later crop.

Pre-harvest infestation rates do not differ for completely closed pods compared to pods with some kind of opening for both field samples. This shows that *A. obtectus* is fully capable of penetrating and ovipositing into closed pods, and infestation of open pods is probably incidental. This is supported by Jarry & Chacon (1983) who found that *A. obtectus* sometimes makes an oviposition orifice in already opened pods. This behaviour of *A. obtectus* is not known by most farmers, but is essential to their control practices of *A. obtectus*. There is an urgent need to educate farmers and help them adapt harvest technology to reduce pre-harvest infestation.

In the study area, natural pre-harvest infestation rate in dry mature pods was 6.9 % infested bean seeds (during Masika 2003). According to Baier & Webster (1992) this is well above the economic damage threshold level of 4%, and therefore control strategies are needed to minimise economic losses. In wilting yellow pods (stage R9A) this infestation rate was reduced to 2.5% infested beans and reduced further to no infestation in yet less mature pods (stage R8B). Late bean harvest resulted in increased infestation and contamination levels in the dry mature pods (stage R9B), because of prolonged exposure time, but not in less mature pods (R9A). This is confirmed by other authors (CIAT, 2001, Olubayo & Port, 1997, Jarry & Chacon, 1983). Farmers would therefore reduce infestation significantly by harvesting as soon as first pods reach the R9A stage, and dry the beans in a place free of bruchids.

In conclusion, this study shows that *A. obtectus* is in its own right a field pest, well adapted to infest growing beans in the field, as well as being able to reproduce in stored bean seed. More in depth research on the insect's ecology outside stores is needed. Farmers need to be made aware of the start of infestation by *A. obtectus*. If farmers knew that their beans were infested in the field before harvest, they could reduce damage to their beans by an early harvest without costs and without the use of pesticides that pose the danger of residual effects. Farmer training programs are urgently needed to educate them on storage pests. They can then take simple measures to reduce storage losses.

Effectiveness of four indigenous botanicals for the management of *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania

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ABSTRACT

This study evaluates the effectiveness of four botanicals at a rate of 1.5 kg per 100 kg beans against *Acanthoscelides obtectus* and *Zabrotes subfasciatus* under both, laboratory and farm conditions. In the laboratory, *Chenopodium ambrosioides*, applied as powder or as whole leaves, was the most effective, with 100% mortality of adult insects in less than three days and no progeny. A reduced amount of *C. ambrosioides* (about 200 g per 100 kg of beans) still resulted in a mortality of 100% in the first 24 hours. *Tagetes minuta* applied as powder also increased mortality and reduced oviposition and progeny significantly. The other treatments - *T. minuta* applied as leaves, and *Azadirachta indica*, *Cupressus lusitanica* and *Phaseolus vulgaris* leaves applied as powder or as whole leaves - had no significant effects upon mortalities, oviposition rate, or progeny production compared with a control with no additions. When the rate of application was increased to about 8.3 kg per 100 kg beans, there was a slight increase in mortality using *T. minuta* and *A. indica*, but not with *C. lusitanica* or *P. vulgaris*. An additional trial with *C. ambrosioides* from different collections and with plants in different developmental stages revealed that there are considerable variations in the efficacy of the treatment. Further studies need to be conducted to clarify the active substances, and to establish the most effective genotype of *C. ambrosioides*, the best harvest time, and the best plant parts to use.

In the on-farm trials, *A. indica* seed powder was the most effective treatment, followed by leaf-powder of *C. ambrosioides* and *C. lusitanica* and *T. minuta*. All treatments were significantly more effective than the control in reducing the numbers of live insects; they also reduced numbers of damaged beans and increased germination rates after 5 months of storage. Farmers' evaluation of the treatments just after the trials and five years later is reported.

Keywords: Stored beans, *Phaseolus vulgaris*; bruchids, *Acanthoscelides obtectus*, *Zabrotes subfasciatus*; botanicals, *Azadirachta indica*, *Chenopodium ambrosioides*, *Tagetes minuta*, *Cupressus lusitanica*; toxicity; progeny; farmers' storage practices

¹ Paul UV, Lossini JS, Edwards PJ, Hilbeck A, submitted 2007, Journal of Stored Products Research.

INTRODUCTION

Improvement of bean production and storage will enhance sustainable development in Eastern and Southern Africa in many ways. Being a major staple crop, it is the second most important source of human dietary protein and the third most important source of calories (Pachico, 1993). Beans are also an important part of the economy: in 1996/97, annual production of pulses in Tanzania was estimated at 374,000 tons, of which 80% is thought to be common beans. In the Arusha region alone, bean production is approximately 16,000 tons. The export value of pulses in the Arusha region in the year 1995/96 was 3 million US dollars (Mashamba, 1998).

A major problem in attempting to increase the supply of beans in rural and urban households is high losses during storage caused by two species of bruchids: *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman (Coleoptera: Bruchidae). Average dry weight losses during storage have been estimated at between 10 and 40% in average, but where management is poor, losses can be well above 50% (Kiula & Karel, 1985; Lima, 1987). Beans with multiple emergence holes of bruchid beetles and emitting a characteristic pungent odour are useless for consumption and have no commercial value (Giga et al, 1992). There is a need, therefore, to investigate environmentally acceptable methods for protecting beans against bruchids during storage.

A study on bean losses during on-farm storage in eastern and southern Africa revealed that all farmers used at least one direct method to protect their beans against bruchid attack. Some farmers used more than one method at the same time: 68% of farmers removed insects by spreading their beans in the sun to dry while 47% used commercial insecticides, 26% mixed ash with their beans and 20% added fine soil; 7% mentioned that they use botanicals, and 2% used some other practice. But most farmers still reported a total commodity loss after 4 - 5 months of storage (Giga et al, 1992). During the field surveys in the area of study, some farmers were observed to be innovative in designing additional storage control practices (Paul & Lossini, unpublished). These included exposing beans to smoke, impregnating the storage bean sacks with hot chilly peppers or goat pellets, and mixing seed beans with kerosene or fungicides used in coffee plantations. The efficacy of such measures has not been proven and toxicity to humans could be a problem. Botanicals, on the other hand, could provide an under-utilized but more effective alternative to these concoctions; these plant-derived materials have the advantages

that farmers can grow them at very low costs and know about their potential toxicity because most of them are used as local medicines. In addition, more complex preparations such as combinations of several substances present in botanicals are less likely to become ineffective because of the evolution of resistance (Regnault-Roger & Hamraoui, 1993; Regnault-Roger et al, 1993).

There are many publications on different botanicals used in storage against different storage insect pests (Golob et al, 1999). Although farmers have considerable traditional knowledge on botanicals, most scientific studies have only evaluated their efficacy in the laboratory. Such studies include the fumigant activity and/or contact toxicity of botanicals to the various life stages of the insects of extracts or dried plant material, and also their repellent and oviposition deterring properties. Some of these studies have used specific isolated components, while others have used crude extracts or powdered plant material. This variety of approaches leads to problems of interpretation, since the insecticidal activity of specific compounds such as essential oils is not necessarily linearly correlated with the content of their main constituents. Very often, the LC₅₀ of crude oils is lower than that found for each constituent by itself (Papachristos et al, 2004), but the studies report results for the most effective form of extract (Boeke et al, 2001). None of these approaches account for the reality as experienced by small scale farmers, who can only use simple methods for preparing botanicals (dried materials, possibly in powdered form).

Recent publications stress the importance of comparing laboratory and field studies for storage trials (Kestenholz et al, 2007). This study evaluates the insecticidal properties of four botanicals under farm and laboratory conditions. Two of these - neem, *Azadirachta indica* A. Juss (Meliaceae), and wormseed, *Chenopodium ambrosioides* L. (Chenopodiaceae) - are known in north-eastern Tanzania as medicinal plants but have not been used traditionally by farmers; however, *A. indica* is well known for its insecticidal properties and several industrial products containing *A. indica* extracts are available (Chiasson et al, 2004a; Isman, 2006). Farmers know it for its medicinal properties (locally called mwarobaini (Swahili), which means the tree that cures forty illnesses) (Dr. Ulicky, personal communication). In the laboratory trials it was decided to use *A. indica* leaves in spite of known lower concentrations of the active component azadirachtin compared to seeds. This was necessary not to introduce 'oil film on seed' as another factor of storage protection (Schoonhoven, 1976). However, in the on-farm trials, we used crushed seeds. This inconsistency

was due to the demand of the extension services to promote neem seed as grain protectant. Chenopodium ambrosioides is also known to some farmers, who call it mangunu (Meru) or ol'kukunu (Maasai), both names meaning that it smells like crushed bedbugs (Cimicidae). Chenopodium ambrosioides and other Chenopodium spp. grow in East Africa and are used in small doses against intestinal worms, stomach aches, constipation, headaches, colds and liver diseases (Kokwaro, 1993). It has been used successfully in storage elsewhere (Golob et al, 1999; Tapondjou et al, 2002). The other two botanicals, cypress, Cupressus lusitanica var. benthamii Miller (Cupperaceae) and marigold, *Tagetes minuta* L. (Asteraceae) are traditionally used in the area for seed storage. Many highland farmers in Arusha apply C. lusitanica for stored maize and beans, and report that they first saw it used in this way in bags coming from Kenya (probably from the ethnic groups Kikuyu and Kamba) (Paul & Mkalimoto, unpublished). There are a few publications indicating that the essential oils derived from *Cupressus* spp.are moderately effective in protecting stored seeds against insect pests (Stamopoulos, 1991; Tapondjou et al, 2005). Tagetes minuta is also widely used by farmers both in the high and low lands (Paul & Mkalimoto, unpublished). Several studies have been written on the use of extracts of T. minuta (Boeke, 2004; Keita et al, 2000; Weaver et al, 1994b), and these also report a reasonable high level of effectiveness.

In this study dried plant material was used, since this is an easy mode of application for farmers to adopt. Trials were conducted in the laboratory and in the field under local conditions, and farmers were given an opportunity to evaluate the different treatments in their own environment and by their own standards. We focussed on two species of bruchids, *Acanthoscelides obtectus* and *Zabrotes subfasciatus* that are the most destructive storage pests for beans in Tanzania. These species often occur together (Abate & Ampofo, 1996), but their relative abundance can change over time because of slightly different optimal living conditions (Schoonhoven, 1976). The life cycles and ecology of the two species are similar; however, an important difference between them from a practical point of view is that *A. obtectus* scatters its eggs freely among the beans, without attaching them to the testa of the bean, while *Z. subfasciatus* larvae bore directly into the bean, and are therefore not or only minimally exposed to the surrounding of the beans, while *A. obtectus* larvae move freely among the beans and search for a place

where two beans touch and they bore into the bean close to that area because it gives them additional leverage to enter the bean (Howe and Currie, 1964; Labeyrie, 1962; Zachariae, 1958). Consequently, physical methods such as sieving the beans regularly reduce numbers of *A. obtectus* more than those of *Z. subfasciatus*. On the other hand, host plant resistance (with the resistance factor arcelin) works against *Z. subfasciatus* but not against *A. obtectus* (Cardona & Kornegay, 1999; Minney et al, 1990). It can be assumed that similar differences between the two species exist for most control methods. As they infest the same storage facilities (or even bean), a control method needs to control both species simultaneously.

The objectives of this study were (i) to assess the efficacy against *A. obtectus* and *Z. subfasciatus* of four locally available botanicals which are traditionally used for medicinal purposes, and (ii) to find practical options for farmers to protect their beans safely and effectively.

MATERIAL AND METHODS FOR THE LABORATORY TRIALS

Insect specimens

Both species were reared at 20±2 °C, 50±15% r.h. and a 12 h photoperiod. The insect cultures were started from natural populations: maturing bean pods from different cultivars were collected from several farmers' fields in the Arusha and Kilimanjaro regions and kept in ventilated jars until the adults emerged. The young adults were put into ventilated jars with dry beans (*Phaseolus vulgaris*, type Calima, cultivar Lyamungo 85 and 90). Pest-free beans were used for rearing. This was achieved by freezing the beans for two weeks and subsequently drying them at room temperature for one hour. The insects were reared according to (Schoonhoven, 1976). The population was maintained for about one year before the trial.

Plants

Calima type dry beans (Cultivar Lyamungo 85) were cultivated at the Selian Agricultural Research Institute (SARI; 3^o22'S, 36^o37'W, elevation 1387masl) and frozen for at least two weeks to exclude any foreign infestation. The beans were dried for one hour at ambient room temperature before used in the trial.

Plant material was collected in late May, whenever possible before noon. Leaves of *P. vulgaris* (bean) were collected from Calima-type beans. Whole young plants of *T. minuta* (beginning to flower) and young leaves of *C. lusitanica* were collected at Olasiti Village in Arumeru District (1350masl), very close to Arusha town. Mature

leaves of *A. indica* were collected from the only mature tree in Nduruma Village (1000masl, about 15 km south east of Arusha town); and whole young plants of *C. ambrosioides* (vegetative state) were collected from Mzimuni Village, Kichangani Hamlet on irrigated land (900masl, about 20 km south east of Arusha town). All these materials were dried in the shade at ambient temperature for two weeks and then placed into well-sealed glass jars until used for the experiment. Plants were identified at the Tanzania National Herbarium in Arusha and checked at the herbarium of the University/ETH Zurich where reference samples are deposited (Agnew, 1974; Beentje & Smith, 2000; Beentje & Ghazanfar, 2003; Beentje & Ghazanfar, 2005; Hedge et al, 1997; Styles & White, 1991; Turill & Milne-Redhead, 1954; Vidakovic, 1991). Just before the experiments began, the dry material of the test botanicals was prepared in two ways. One part was ground in a ceramic mortar, sieved first through a sieve of 2 mm, and the fraction < 0.25 mm was determined by a further sieving. The other part was used as intact leaves or leaf pieces, as some breaking for weighing and putting into the vials for the experiment was unavoidable.

Bioassays of main trial

Twenty beans were placed in a glass vial of about 18±2 ml volume. For the first seventeen replicates with A. obtectus and the first eighteen replicates with Z. subfasciatus, 0.18±0.01 g of the test botanicals was added to each vial. For the remaining eight replicates (two with A. obtectus and six with Z. subfasciatus), 0.15±0.01 g of botanical was used because the beans came from a different harvest and were much smaller. The beans were weighed in order to assess whether bean size influences the efficacy of treatments. The botanical material was either in a leafy or powdery form. As standard and control, beans with *P. vulgaris* leaves (in powdery or leafy form) and beans without any addition were used. Young adult insects less than 48 h after emergence were used in all trials. Four unsexed A. obtectus or a male/female pair of Z. subfasciatus were placed into the vial. The vials were sealed with a rubber lid, placed into an open box, and kept at 20±2℃, 50±15% r.h. and a 12 h photoperiod. On four days (18 June, 9 July, 14 July and 16 July, 2004), as many replicates as possible were added with the newly emerged insects. These were in one case only one replicate and at most thirteen replicates. After 3, 5, 7, and 14 d, the vials were emptied onto a plastic sheet, and the living and dead insects counted (an insect was considered dead if it did not move the antenna or the legs when touched twice with tweezers). Eggs were counted on day 18, when it was assumed that no more eggs would be laid (Parsons & Credland, 2003). All insects, beans and botanicals were carefully returned to the vials after the assessments. On day 28, all adult *A. obtectus* were removed, placed in the freezer for 2 d, and the sex was determined. Starting on day 56 after placing the insects into the vials, each vial was checked three times a week for emerging adults.

Bioassays of additional trials

In a trial set up on 22 July, the vials were filled with different amounts of nonpowdered botanicals (*C. ambrosioides*: 0.1 g, 0.05 g and 0.025 g/vial; all other treatments: 0.25, 0.5 and 1 g/vial). No beans were added as only mortality was assessed. Two unsexed *A. obtectus* or a pair of *Z. subfasciatus* (one male and one female) were put into the each vial. Mortality was checked on day 1, 2, 5, 7 and 14.

In a further trial set up on 27 August, *C. ambrosioides* from two different locations and development stages were compared. The plants were collected two weeks prior to the trial in Arusha town, beside the main road, and in Kichangani Hamlet of Mzimuni village (where the *C. ambrosioides* for the main lab oratory trial was collected). In both places, plants in the vegetative stage (i.e. neither flowers nor seeds) and plants in the reproductive stage (i.e. with flowers and seeds) were collected. The material was dried as described above, and twenty beans and 0.1 g of botanical as whole leaves placed in each vial. Four unsexed *A. obtectus* were added and mortality was observed on day 1, 2, 5, 7, and 14 after the beginning of the trial. As a control, the data from the main trial were used (see above).

Statistical analysis

The opportunistic method of preparing as many replicates as possible resulted in an unbalanced trial design. The data violated the assumptions for normal distribution and homoscedasticity. Therefore, the variance was tested with the Kruskal-Wallis test for the factors botanical (control, *P. vulgaris* leaves, *C. lusitanica* leaves, *A. indica* leaves, *T. minuta* leaves, *C. ambrosioides* leaves) and treatment form (control, powder or leaves) (Mas & Dietsch, 2003). For the data on oviposition and *A.obtectus*, the powdery application form was excluded because it was impossible to count the eggs accurately in these treatments. Then post-hoc multiple comparisons with Dunnet's T3 test (Anonymous, not dated) were performed. For the data on mortality until day seven and on the numbers of emerged adult insects, a one-factor analysis using the combination of the two factors mentioned above was performed. This resulted in eleven treatments to be compared. A possible effect of a) the weight of the beans on oviposition, b) the amount of botanical on mortality, and c) the number of females on oviposition, was examined using ANCOVA as non-conservative test that would allow discovering any irregularities in the trial set-up or with systematic errors. All results were compiled using SPSS 9.0 and Microsoft Excel 2003.

MATERIAL AND METHODS FOR THE ON-FARM TRIALS

Farmer selection

Seven villages were selected to represent the variety of climatic, natural and cultural diversity of the Arumeru District in North-eastern Tanzania (Fig. 1 and Table 1).

Baraa is a suburban farming village with a mixed ethnic population (Meru and Arusha). Kikatiti is an important market place (especially for animal trade) and visited by farmers from afar. Kimundo is the home village of the old Meru Chiefs (Mangi). Kisimiri was a wheat estate owned by foreign settlers until the land was given back to the Meru farmers. Mzimuni was part of a sisal estate and, although many workers from very different ethnic groups remained after the estate was dissolved, the Arusha and Maasai influence the culture greatly. Olmotonyi is close to the Meru forest plantations. Former wheat estates were located in Oloitushula. In each village, a community meeting was held at which storage practices were discussed and two farmers were selected who offered their crops and storage facilities for the trial. These farmers were interested in collaborating just because they wanted to find out better pest control measures for their stored crops and we assured them that we would compensate increased losses if they would occur.



FIGURE 1: Map of the Arumeru District in Tanzania and the locations of the on-farm trials. Shaded area give an approximate extension of the area where the Meru people live and non-shaded area where the Arusha people live. Adapted from (Spear, 1997) and (Semu et al., 1992).

TABLE 1: Climatic and socioeconomic characteristics of the seven research villages in the Arumeru

 District (Tanzania).

Remark: Information is from various local sources and informants. The population density and growth rate are calculated with the results of the population census from 1978, 1988 and the area at ward level. The actual migration movements were taken into consideration.

Village	Altitude m.a.s.l.	Rainfall mm/ year	Ethnicity	Population density Growth/ year	Village formation (Year)
Baraa	1200 – 1500	1000 – 1300	Meru/ Arusha	400p/km² 2% increase	Traditional Arusha town
Kikatiti	1000 - 1200	500 - 800	Meru/ mixed	70 p/ km² 3.5% increase	Traditional (1950 ?)
Kimundo	1200 - 1600	1200 - 1500	Meru	300 p/ km² 2% increase	Traditional (1600)
Kisimiri	1400 - 2000	500 - 800	Meru	30 p/ km² 3% increase	New (1960) Former estate
Mzimuni	900 - 1100	400 - 600	Arusha/ mixed	70 p/ km² 3.5% increase	New (1960 ?) Former estate
Olmotonyi	1500 - 1800	800 - 1200	Arusha	390 p/ km² 2.5% increase	Traditional (1880)
Oloitushula	1400 - 1800	500 - 800	Arusha	70 p/ km² 3.5% increase	Traditional (1910)

Plants

The selected farmers provided a sample of their stored beans for an initial check on infestation. They also provided 60 kg of beans harvested the previous season for the trial. Each collaborating family offered the bean variety they cultivated; these included: Maasai red, Canadian Wonder, Kablanketi, Jesca, Calima types and Lyamungo '90. During the month of August, whole flowering plants of *T. minuta* and small branches of C. lusitanica were collected close to the town of Arusha (in similar conditions as in the laboratory trial), seeds of A. indica were collected from Hai (60 km east of Arusha, 800 m.a.s.l.) and Same (250 km south-east of Arusha, 300 m.a.s.l.), and whole plants with mature seeds of C. ambrosioides were collected in Mzimuni (same place as in laboratory trial) and Arusha (not exactly the same places as in laboratory trial). All leaves were dried in the shade for 14 d and then ground to powder. A. indica seed was dried and ground without shelling of the seed. In this trial it was decided to use seeds instead of leaves (as in the laboratory trial), as the known active component azadirachtin is found at a higher concentration in seeds than in leaves. But this meant to collect the seed from outside Arusha region. All dried botanicals were kept in sealed jars until used for the trial.

Trial protocol

In September, four lots of 15 kg beans were weighed on each farm and mixed with one of three dried botanical (half the farms used *T. minuta*, the other half used *C. lusitanica*). The rates were 10 g/kg for *C. ambrosioides*, and 15 g/kg for all others. As a control, we used the fourth lot of 15 kg of beans without adding any botanical. The thoroughly mixed beans were divided into three bags of 5 kg made from jute, labelled, and stacked randomly at the designated storage place which the farmer had previously selected. Infestation occurred naturally. We took a sample of about 100 g from the centre of each bag each month for 5 months (resulting in a total of five samples collected over a half a year period), and counted the dead (no movements), morbid (moving antennae but not legs) and live adult insects, and assessed percent of damaged beans (with one or more emergence holes). For a germination test with each sample from Mzimuni (no *C. lusitanica* treatment), 3x10 seeds were placed into closed Petri-dishes with wet filter papers and kept in a dark room. The beans were regularly checked and the papers were kept humid. On the eighth day, all germinating seeds (visible germ) were counted.

Analysis:

Means of the percent of damaged seeds were calculated for each treatment on each farm and ranked according to severity of damage (1: least damage, 4: most damage). Thus the varying levels of infestation between the farms were excluded to influence the analysis. The data was then analysed with the Kruskal-Wallis-test for differences between the treatments and, where significant, Dunnett's T3 test was used for the post-hoc separation of the means. The analysis was performed using SPSS 9.0 and the graphs were calculated using Microsoft Excel 2003.

RESULTS FOR LABORATORY TRIALS

Influence of weight of beans and amount of botanical used

The average weight of beans per vial, which varied between 4.1 and 13.5 g, had no significant influence upon oviposition by either of the two storage pests during the first 18 d (P=0.677, F= 0.174, DF=1 for *A. obtectus* and P=0.343 F=0.906, DF=1 for *Z. subfasciatus*). Also, neither the amount of botanical used nor the ratio between amount of botanical to weight of beans showed any significant effect on mortality (data until 7 d after application of treatments: *A. obtectus*: P=0.884, F=0.021, DF=1

Number of females per vial

Although *A. obtectus* adults were not sexed before transferred to the vial, all vials contained at least one female. As the young adult beetles were already up to 48 h old, most of the females had probably been fertilized before they were put into the vial for the trial (Parsons & Credland, 2003). Thus offspring were produced even in one vial that contained only four females. An analysis with ANCOVA showed that the number of females in the vials did not significantly influence the number of eggs laid by day 18 (P=0.681, F=0.171, DF=1), leading to the conclusion that reproductive output was more restricted by factors such as space or competition than by the number of females. Thus, all results (see section 4.5 to 4.7) are presented as numbers of progeny per vial rather than per female.

Particle size distribution

The ground leaves of *Ph. vulgaris* contained $51\pm9\%$ of particles smaller than 0.25 mm. The equivalent percentages for the other treatments were $37\pm30\%$ for *C. ambrosioides*, $29\pm21\%$ for *C. lusitanica*, $22\pm16\%$ for *A. indica* and $17\pm9\%$ for *T. minuta.* The results show no consistent effect of particle size distribution on mortality.

Mortality of beetles as influenced by treatment and form of treatment

In the main trial with about 1.5 kg of botanical per 100 kg of beans, a difference between the treatments was graphically obvious on day 3 after application of treatments and was maintained until day 14 (Fig. 2). Statistically, mortality until day 7 was significantly influenced by treatment and form of treatment (see details in Fig. 3): The *C. ambrosioides* treatment always led to 100% mortality of both *A. obtectus* and *Z. subfasciatus* within three days (P<0.001). The use of *T. minuta* powder resulted in a significantly higher mortality of both species (60%, P=0.002 for *A. obtectus* and 74%, P<0.001 for *Z. subfasciatus* until day 7 after application of treatments) than in the control (6% for both species also until day 7). However, when whole leaves of *T. minuta* were used there were no significant differences in mortality rates compared with the controls (2.5%, P=1 for *A. obtectus* and 8%, P=1 for *Z.* subfasciatus until day 7). None of the other treatments (both leaves and powder of *Ph. vulgaris, C. lusitanica* and *A. indica*) had any significant effect upon the mortality of either species.



FIGURE 2 : Mean accumulated mortality in percent for *A. obtectus* (a and b) and *Z. subfasciatus* (c and d) after 3, 5, 7, and 14 days of exposition to five botanicals (*Ph. vulgaris, A. indica, C. lusitanica, T. minuta* and *C. ambrosioides*) either as powder or as whole leaves and the control (no addition).

In the additional trials with reduced amounts of *C. ambrosioides* powder (from 0.18 g to 0.025 g per vial), all test-insects of both species died in 24 hours. However, experiments to assess other ecotypes and developmental stages of *C. ambrosioides* produced no conclusive results. The leaf-material from Arusha town induced a higher mortality than the material containing fruits and flowers of the same origin. In contrast, for the material from the Mzimuni region, the fruit/flower- material induced a higher mortality than the leaf material (Fig. 4).

Increased amounts of non-powdered *T. minuta* (to 1 g per vial or about 8.3 kg per 100 kg beans) increased mortality of *A. obtectus* slightly, but were not conclusive for *Z. subfasciatus* (Fig. 5e and 5f). On the other hand increasing doses of *C. lusitanica* raised mortality slightly in *Z. subfasciatus* but not in *A. obtectus* (Fig 5c and 5d).

Increasing the dose of *A. indica* leaves (to 1 g per vial or about 8.3 kg per 100 kg beans) raised mortality of both species slightly (Fig. 5g and 5h).

Increasing the dose of *Ph. vulgaris* leaves up to 1 g per vial (equal to 8.3 kg per 100kg beans), had no effect on *A. obtectus* mortality compared to the control, but possibly increased mortality in *Z. subfasciatus* slightly (Fig. 5 a and b).



FIGURE 3: Mean mortality in percent (±SE) for *A. obtectus* and *Z. subfasciatus* until day 7 after application of treatments for five botanicals (*Ph. vulgaris, A. indica, C. lusitanica, T. minuta* and *C. ambrosioides*) and the control. Kruskal Wallis: χ^2 = 89.98, DF=10, P<0.001 for *A. obtectus* and χ^2 = 100.31, DF=10, P<0.001 for *Z. subfasciatus*. Significant differences between treatments (of the same insect species) are marked with different letters below the column. Dunnett's T3 test (P<0.05).



FIGURE 4: Mean accumulated mortality in percent for *A. obtectus* after 1, 3, 5, and 10 days of exposition to *C. ambrosioides* leaves from two different collection sites and two different maturity stages. Data for hypothetical control (no added botanicals) taken from the main trial.



FIGURE 5: Mean accumulated mortality in percent for *A. obtectus* (a, c, e, and g) and *Z. subfasciatus* (b, d, f, and h) after 1, 2, 5, 7, and 14 days of exposition to 0.25, 0.5, and 1 g/vial of leaves of four botanicals (*Ph. vulgaris* (a and b), *C. lusitanica* (c and d), *T. minuta* (e and f) and *A. indica* (g and h).

Oviposition

It was difficult to count the eggs of *A. obtectus* in the treatments using powdered plant material, and only the data obtained using whole leaves were analysed. No eggs were found in the vials treated with *C. ambrosioides* whereas the control vials contained a mean of 27.1 eggs per vial (p<0.001). In contrast, oviposition in the treatments with *T. minuta* (30.7), *C. lusitanica* (26.3), *A. indica* (26.0) and *Ph. vulgaris* (19.7) was not significantly different from that in the control vials (Fig. 6).

Because the eggs of *Z. subfasciatus* are glued to the bean testa, they are easily visible, and so data could be collected for both the powdered and whole leaf treatments. There were no eggs laid in vials treated with *C. ambrosioides* whereas the controls contained a mean of 15.2 eggs per vial. The use of *T. minuta* (5.4) resulted in a lower oviposition than using *C. lusitanica* (10.3), *A. indica* (12.9) or *Ph. vulgaris* (17.8) (Fig. 6). There was no significant difference (χ^2 =3.473, P=0.176, DF=2) between the application as whole leaves or as powder (10.2 and 8.9 eggs per vial respectively).



FIGURE 6: Mean numbers of eggs per vial (±SE) laid by *A. obtectus* and *Z. subfasciatus* after application of five different botanicals (*Ph. vulgaris, A. indica, C. lusitanica, T. minuta* and *C. ambrosioides*) as whole leaves (*A. obtectus*) and as powder and leaves (*Z. subfasciatus*), and a control until day 18 after application of treatments (exact amounts and methods are described in chapter 2.3). Kruskal Wallis: χ²= 28.77, DF=5, P<0.001 for *A. obtectus* and χ²= 56.69, DF=5, P<0.001 for *Z. subfasciatus*. Significant differences between treatments (of the same insect species) are marked with different letters below the column. Dunnett's T3 test (P<0.05).</p>

Emergence of F1 adults

The mean number of adults emerging from the control vials was 18.40 for *A. obtectus* and 6.19 for *Z. subfasciatus*. In contrast, no adults emerged from vials treated with *C. ambrosioides*. *Tagetes minuta* applied in powdery form resulted in significantly fewer emerging adults per vial than in the control (mean of 2.48 emerged adults for *A. obtectus* and 0.20 emerged *Z. subfasciatus*), but no other treatments were significantly different from the control (Fig. 7).

The mean number of *A. obtectus* adults emerging per egg was lower in the treatment with whole *T. minuta* leaves than in controls (0.29, N=10 v. 0.82, N=20), and also lower than in the other treatments (*Ph. vulgaris*: 0.67, *A. indica*: 0.69, *C. lusitanica*: 0.88, N=10 for each treatment). Also, there were fewer *Z. subfasciatus* adults emerging per egg in the treatments with powdered *T. minuta* leaves than in the control (0.00, N=3 v. 0.4, N=15) and the other treatments (all higher emergence per egg than the control). However, none of these differences were statistically significant due to the low number of vials with eggs.



FIGURE 7: Mean emergence of F1 generation per vial (±SE) of *A. obtectus* and *Z. subfasciatus* (where parent generation was exposed during oviposition to five botanicals (*Ph. vulgaris, A. indica, C. lusitanica, T. minuta* and *C. ambrosioides*) in powdered form or as whole leaves for 28 days; botanicals were left in the vials during development of F1 generation. Exact amounts and methods are described in chapter 2.3). Kruskal Wallis: χ^2 = 60.71, DF=10, P<0.001 for *A. obtectus* and χ^2 = 65.25, DF=10, P<0.001 for *Z. subfasciatus*. Significant differences between treatments (of the same insect species) are marked with different letters below the column. Dunnett's T3 test (P<0.05).

Development time from oviposition to first emergence

No significant differences between the treatments were found in the time until the first F1 adult beetle emerged. Development times ranges from 59 d to 103 d, with an average of 73 d for *A. obtectus* and 75 d for *Z. subfasciatus*.

RESULTS FOR ON-FARM TRIALS

In all five assessments, more damaged beans were found in the non-treated samples (mean of 67% 5 months after application) than in the samples treated with powdered leaves from *T. minuta*, *C. lusitanica*, *C. ambrosioides*, or with *A. indica* seed-powder (Fig. 8a).





- a) Mean percent of damaged beans (at least one emergence hole visible on bean).
- b) Mean numbers of live, moribund and dead insects per sample of beans (100 g).
- c) Mean live, moribund and dead insects as percentage of all insects present in sample.

The analysis showed that *C. ambrosioides* protected the beans significantly better from damage for the whole 5 month period compared to the control. *Azadirachta indica* seed treated beans also showed significantly less damage than the control after the first month. Treating beans with *T. minuta* reduced bean damage significantly after 3 months of storage until the end of the trial. Beans treated with *C. lusitanica* were consistently less damaged than the control, but the difference was only significant for the third month after treatment (Fig. 8a).

There were fewer insects (alive, morinbund and dead together) in samples with *T. minuta, C. lusitanica* or *C. ambrosioides* than in the untreated control and in samples treated with *A. indica*-seed powder (Fig. 8b). There were more insects that were dead and moribund in the samples treated with leaves of *C. ambrosioides* and *A. indica* seed-powder than in the untreated control, the *T minuta* or *C. lusitanica* treated samples. All four botanicals resulted in a high percentage of moribund pest insects one and two months after application of the treatments. The proportion of moribund insects decreased during months 3 to 5 for beans treated with *T. minuta* and *C. lusitanica*, but it remained relatively high for beans treated with *C. ambrosioides* and with *A. indica* seed powder. The proportion of dead insects was lower than the control for beans treated with *T. minuta* or *C. lusitanica*. For the beans treated with *C. ambrosioides* the proportion of dead insects was very similar to that of the non-treated control. And for *A. indica* treated beans, the proportion of dead insects was higher than in the control (Fig. 8c).

Germination remained close to 100% for the first 3 months in all treatments and the control, but then declined with time. Less than 50% of the beans from non-treated samples germinated after 5 months of storage compared with 60% - 70% for the treated samples.

DISCUSSION

The laboratory trials revealed a very high toxicity of *C. ambrosioides* to *A. obtectus* and *Z. subfasciatus* that was not dependent on the form of application (dried whole leaves or powdered leaves). All trial insects died within 24 h in all main trial replicates and at all doses (the lowest being equivalent to 200 g of botanical per 100 kg beans).

Leaves of *T. minuta* were moderately toxic to *A. obtectus* and *Z. subfasciatus* when applied as a fine powder, but did not increase mortality compared to the control when applied as whole leaves.

The on-farm trials suggested that *A. indica* seed powder is effective in protecting stored products for up to 4 months (or for two to three generations of insects). However, *C. ambrosioides*, *T. minuta* (both dried and ground young plants) and *C. lusitanica* (leaves in powdered form) also have a good potential for short term storage (up to 2 months or one to two generations of insects).

Variable but reasonably high mortality of A. obtectus was obtained using C. ambrosioides plant material from two different places and of different developmental stages. However, it is not clear from our results whether flowering/ fruiting plants or vegetative plants are more toxic. Some authors reported that C. ambrosioides fruits but not leaves have insecticidal activity against Sitophilus zeamais (Tavares & Vendramim, 2005). Kayitare & Ntezurubanza (1991) found that leaves of Chenopodium procerum (a close relative to C. ambrosioides) were more toxic against A. obtectus than flowers, but they also reported that flowers of an unidentified Chenopodium species were more toxic to Z. subfasciatus than those of C. procerum. The essential oil composition of C. ambrosioides is varying greatly between different origins of the plant material: ascaridole derivates are major components and and constituted 60% in a study from Madagascar (Cavalli et al, 2004), 50% in a nother study from Iran (Omidbaigi et al, 2005) but only 7% in a study from India (Deepti-Gupta et al, 2002). There might be differences of the chemical composition in regard to different plant parts and/or different plant stage as found for C. lusitanica (Kuiate et al, 2006) and T. minuta (Bikram-Singh & Virendra-Singh, 2002). Or, it could be a genetic variation as found in *T. minuta* (Alok-Krishna et al, 2005). However, it is concluded that *C. ambrosioides* is a very promising candidate for use as an insecticidal plant, but further investigation is needed into the combination and concentration of the active insecticidal substances and their toxicity in different plant organs of different ages and origins.

The farmers in our experiments who used *C. ambrosioides* complained about the pungent smell and the bad taste of the cooked beans. This could be a question of dosage, as in South America, *C. ambrosioides* is used as a flavouring. It is also reported that bean dishes flavoured with *C. ambrosioides* keep fresh for longer due to bactericidal effects of *C. ambrosioides* (Logan et al., 2004). *Chenopodium ambrosioides* is used for diverse medicinal purposes, but one author reported that its use as treatment of intestinal worms was discontinued because of human fatalities due to mammal toxicity of ascaridole (MacDonald et al, 2004). Experiments with mice

have shown a relatively low acute toxicity (LD_{50}) of more than 1 g/kg oral dose (Olajide et al, 1997), but a chronic toxicity with physical abnormalities of lungs, stomach lining and kidneys after 6 weeks (Amole & Izegbu, 2005). There are also reports on cytotoxic and genotoxic effects on human lymphocytes (Gadano et al, 2006). Therefore, an in-depth study on effects of *C. ambrosioides* on humans is essential before promoting its use as an insecticide for food crops. However, a commercial product containing *C. ambrosioides* extracts is already available and has been shown to be effective against insects and mites while having a negligible effect on biological control agents (Chiasson et al, 2004a; Chiasson et al, 2004b).

Tagetes minuta was insecticidal as a powder only. This could indicate that *T. minuta* is either a contact insecticide (as the insects were covered with the powder but they probably could evade the leaves), or that the vapour concentration was too low when the leaves were whole. Other authors have found similar effects: for example, *Ocimum canum* leaves were only effective in reducing insect populations when finely powdered (Weaver et al, 1994a). It is unlikely that the particle size itself influenced the results, since mortality did not increase when the other botanicals were applied as a powder. Furthermore, research has shown that dusts are only effective in killing insects through desiccation (Golob, 1997) when the particles are very small; for example, the dose of a hydrophobic fumed silica needed to protect wheat against *Sitophilus granarius* was 10 times higher with a particle size between 15 μ m and 20 μ m than with a particle size of 0.012 μ m (Aerosil R974[®]) (McLaughlin, 1994).

In our laboratory trials, *A. indica* leaves in amounts up to 8.3 kg per 100 kg beans showed little insecticidal properties while the pounded seeds at 1.5 kg per 100 kg beans reduced storage pests significantly in the farm trials (below 10% damaged beans 4 months after application of treatments). The conclusion that neem-seed formulations are more effective against insects than neem-leave formulations is also reported by other authors (Jilani & Malik, 1973; Makanjuola, 1989). Neem-leaves seem to cause a significant reduction in F1 progeny for *A. obtectus* and *Z. subfasciatus* only at a much higher concentration of 50% (v/v) as reported by Chinwada & Giga (1997).

Although the trials were not specifically designed to show attractive or repellent effects, a few conclusions can be drawn from the on-farm trials. They were based on natural infestation of the stored treated beans, and the results are therefore influenced by repellence as the infesting insects had the choice of which bag to infest and could move more or less freely between the different treated bags. *Azadirachta indica* seed powder was less repellent than any of the treatments (except the control), as the *A. indica* treated bean samples contained more total insects than samples from bags with the other treatments in spite of a high mortality and consequently reduced propagation. But literature is not conclusive about the attractive or repellent effect of *A. indica*: an attractive effect of *A. indica* on *Callosobruchus maculatus* has been reported (Boeke, 2004; Boeke et al, 2004) as has a repellent effect against *Callosobruchus chinensis* (Lawati, 2002; Pandey et al, 1986) and a neutral effect against *A. obtectus* (Mazzonetto & Vendramim, 2003).

The reduced numbers of insects in the on-farm trials without an accompanying high toxic effect in *T. minuta*, *C. lusitanica* and possibly *C. ambrosioides* suggests a repellent effect. This is consistent with other literature showing a weak repellent effect of *T. minuta* against *Callosobruchus maculatus* (Boeke, 2004). Cypress was similarly found to be repellent against *Sitophilus zeamais* and *Tribolium confusum* (Stamopoulos, 1991; Tapondjou et al, 2005). It is less clear for *C. ambrosioides* in one study (Mazzonetto & Vendramim, 2003) but not against *Sitophilus zeamais* in another study (Tavares & Vendramim, 2005).

With *C. ambrosioides* treated beans, no eggs were laid by either *A. obtectus* or *Z. subfasciatus* and therefore no adults emerged in these treatments for both species. Therefore, we cannot evaluate the toxicity of this botanical on stages other than the adult insects. Beans treated with powdered *T. minuta* produced fewer adult *Z. subfasciatus* per egg, and beans treated with *T. minuta* leaves produced fewer *A. obtectus* adults per egg. This indicates that *T. minuta* has a toxic effect on the juvenile forms of *A. obtectus* and *Z. subfasciatus*. The only relevant literature found describes an ovicidal effect of kaolin powder aromatised with *T. minuta* essential oil against *A. obtectus* (Keita et al, 2000) and an ovicidal effect of Cypress essential oil against *Callosobruchus maculatus* (Stamopoulos, 1991). We could not confirm this. It has to be established if this difference is caused by using plant powders versus essential oil vapours. Other literature describes *A. indica* as being an oviposition deterrent against *Plutella xylostella* (Facknath), but we know of no studies of the effect of *A. indica* or *C. ambrosioides* on bruchids.

Farmers were interested in doing the experiment in their village. A group of about ten farmers were present when the trial was set up and each month, when the samples were taken, a few farmers came to watch the change in the different bags. In a final evaluation, the farmers were mainly impressed with the results of *A. indica* and *C. ambrosioides*. They said that it was difficult for them to obtain *A. indica* seed and asked if the leaves might be sufficient to protect their beans. This led to the decision for using *A. indica* leaves in the laboratory trials. *C. ambrosioides* was evaluated as a good treatment but it was too smelly and food cooked from beans treated with *C. ambrosioides* was said to be unpalatable. Many farmers wanted to use it for storing seed beans for the following planting season, saying that the smell would deter anybody from using it for food.

An informal evaluation five years after the initial on-farm trials showed that farmers originally participating still preferred *C. ambrosioides* for protecting their seed beans from insect damage. Some farmers also actively promoted the growth of *C. ambrosioides* close to their homestead. For human consumption, most farmers used either dried and powdered *A. indica* leaves or *T. minuta* plants. Farmers in the trial villages who were not part of the farmers' research group did not change their habits and did not know about the use of *C. ambrosioides* in storage. Therefore we conclude that innovations are not likely to spread easily from one farmer to his or her neighbour, but need active promotion, such as small trials set up in individual homes. Also, actively exchanging information across regions about the most effective botanicals could greatly help farmers.

Overall, we consider that this study was a success and offers a model for future research projects. Not only could we confirm scientifically the efficacy of certain botanicals and determine their most effective form (powder or leaves), but farmers benefited directly from the results.

Synthesis

In light of the insight gained during the collaboration with farmers and the experimentation with organic resources for bean pest management, the following theses can be advanced with regard to local organic control practices in Tanzania:

- (1) Farmers use treatments that control pests, but effectiveness and duration of control varies greatly.
- (2) Farmers concentrate their pest management efforts to where it is most effective: more control practices are used in storage than in the field crop.
- (3) Farmers observe, experiment, and adapt production and storage with respect to local conditions.
- (4) Farmers know the damage done by pests, but their knowledge on the pest ecology is limited.
- (5) When farmers understand the lifecycle of the pest in more detail, they gain confidence and are more likely to teach other farmers about their control practices.

The final chapter of this dissertation elaborates these five theses also based on the six years of personal experience of working in North-East Tanzania. Many examples will be viewed through my own set of glasses. At the beginning I thought them to be transparent and objective, but the collaboration with the Tanzanian farmers gave them a new special tinge.

(1) Farmers use treatments that control pests, but effectiveness and duration of control varies greatly.

Farmers worked their farms for generations and developed strategies to manage pests. Obviously, they evaluated the effectiveness of these strategies in their own way to improve them. This means that crop protection aspects of traditional agriculture have evolved with the system and are complex (Bajwa & Schaefers, not

dated). They were often low input systems and operated efficiently, but generally did not produce high yields (Van Huis & Meerman, 1997). On the positive side, pest outbreaks in these conditions were rare. However increasing population pressures are changing this situation rapidly and pest problems are expected to continually increase (Abate et al, 2000). It is in this view that traditional practices should be evaluated for their effectiveness.

During informal surveys and talks with farmers in Arusha, Kilimanjaro and Usambara mountains, many pest management practices were discussed. It became apparent that most farmers used local resources for controlling insects primarily in storage and that practices varied from place to place depending on farming system and availability. A more formal survey (Paul, unpublished) revealed that there was not a distinct difference in knowledge of possible local organic resources in the three areas; most often some sort of botanicals with medicinal purposes were used. Substances used in certain areas but not in others were generally not traditional, but were introduced either through a specific project or related to a cash crop and introduced by commercial advisors. Detailed discussion on different control methods in a community group setting was new for most farmers. Farmers did not always agree on the effectiveness of a specific treatment, and sometimes a practice continued to be used although it was not considered effective. This was mainly due to the lack of better options. Farmers were keen on testing some practices and learning about other options. Most trials combined traditional and new practices.

The evaluated pest management practices against *O. bennigseni* (chapter 4) and storage pests (chapter 6) were not strictly traditional treatments, but chosen in collaboration with farmers on expected efficacy, local availability and ease of use. As mentioned in the respective chapters, urine was promoted by another integrated pest management project (Gesellschaft für Technische Zusammenarbeit, GTZ). It was therefore known in two regions (Kilimanjaro and Arusha), but not in the Usambara mountains. Neem was known as a medicinal plant, but it was promoted for agricultural use by previous researchers from CIAT. Vernonia was suggested by some farmers in the Usambara mountains, as it seemed to be as bitter as neem, and farmers used it traditionally against malaria. It was also known in the other regions and used for medicinal purposes. Tagetes was used traditionally for storage by several farmers in all regions. Cypress was used by farmers in Arusha, but was introduced not long ago possibly through Kenyan immigrants. Chenopodium was

only known by a minority of people (Arusha and Kilimanjaro regions) and used as medicinal herb for stomach problems.

The trials showed that the suggested treatments reduced insect abundance for a limited time ranging from 24 hours (urine in field application against *O. bennigseni*) to about 4 months (neem seed powder against bruchids in on-farm trials). Some were very effective (especially urine against O. bennigseni and chenopodium against bruchids in laboratory trials), others had a limited effect on the target species, such as neem or cypress leaves against storage pests. This limited effect could be inherent to the substance, or dependant on the form of application and/or dose, as shown with tagetes whole leaves and leaf-powder in the storage trials (chapter 6). Astonishingly few studies report on effectiveness of traditional practices in pest management. The only exceptions are well known insecticidal plants such as neem or storage practices. Local practices should be tested more often scientifically in collaboration with farmers. In my experience, some farmers continued to use less efficient treatments, such as cypress leaves in storage, in spite of data showing the limited effectiveness. Reasons ranked from availability, taste preferences and perceived effectiveness. A major draw back was the lack of ways to enhance and prolong effectiveness with simple methods. Future research should focus on such improved preparations.

(2) Farmers concentrate their pest management efforts to where it is most effective: more control practices are used in storage than in field crops.

Farmers were much more aware of local substances that could be used for pest control in storage than substances to be applied on field crops. In Malawi 80% of field pests remained uncontrolled due to lack of knowledge of potential control measures; in contrast 84% of respondents with storage pest problems used one or more control measures (Ross, 1998). Although farmers in Kenya were very knowledgeable about field pests and considered them very damaging, activities to control them were minimal (Chitere & Omolo, 1993). Unfortunately, the authors did not explain this discrepancy.

Possible explanations are that fields can recover and compensate from pest damage, but once harvested one can only lose. Also loss in storage is not limited; if nothing is done for long enough, everything will eventually be consumed by storage pests (Goldman, 1991). Therefore farmers' interventions for storage protection are
generally more extensive than for field pests (Goldman, 1991). The treatments used for the storage trial were more effective than the ones suggested for the field trials. This could be due to more detailed knowledge and experience of farmers in storage or because of the more controlled and enclosed system.

In the field farmers experimented more often with varieties and different crops than with cultural methods and/or inputs (such as pesticides). New varieties and crops are also more often exchanged and disseminated than more complex technologies (Hollenweger & Mkalimoto, 2001).

(3) Farmers observe, experiment, and adapt production and storage in respect to local conditions.

Farmers' practices are developed with experience and close observation, even without a scientific experimental attitude. In the field farmers' practices are often incidental pest management strategies. For instance, farmers did not practice any curative methods against *O. bennigseni*, but they learned that delaying planting resolved much of the problem with this pest, without knowing the exact reason. In Tanzania and Malawi farmers explained that the adult beetles fell from the sky with the first rains and were washed away by heavy rains (Ampofo et al, 2002, Ross, 1998). With insufficient rainfalls (Abate et al, 2000), they had to search for other control methods as an early planting date became crucial for a good harvest. In storage the practices are more often deliberate, and botanicals are commonly used. The reason is that the effect of a deliberate action is easier to asses in a relatively simple storage system than in the very complex field situation. Farmers' practices are locally adapted, and therefore result in different practices for areas with different pest occurrence, such as storing beans in their pods or threshing them, depending if the major storage pest is Z. subfasciatus or A. obtectus, although farmers do not differentiate between the two bruchid species (Giga et al, 1993).

When working with farmers and using organic substances for pest control, farmers started to experiment with those substances in other crops and pests: Neem seed powder was reported successful in reducing diamond back moth in cabbage (though it was also told that it does not have the quick knockdown effect like commercial insecticides). Tobacco products were tried against aphids and even followed up with a visit to the research station, where the farmers evaluated the effects of the different treatments under the microscope. Farmers tried urine against armyworms,

but found it not effective and against lepidopteran pests such as *Helicoverpa* spp, where they reported only little effect. Four to five years after the original trials with seven villages in Arusha, farmers had adjusted some of their practices. Several farmers planted *Chenopodium* spp. in their backyard to have sufficient plant material for their storage needs. And others used the labour intensive treatments learned during the project for higher value crops such as vegetables.

(4) Farmers know the damage done by pests, but their knowledge on the pest ecology is limited.

Pests (or other insects, or natural phenomena) can be divided into four groups according to their importance and ease of observation (Bentley, 1991): Pests considered important that are easily observed are readily known by farmers and more likely to be controlled deliberately. Unimportant but conspicuous insects are known, but do not attract extensive explanations about their origin and are not controlled. Important pests that are not easily seen are more often judged by their damage and attract folkloristic explanations and superstitious management strategies. Unimportant and difficult to observe insects are not known (Bentley, 1991).

Looking at some Tanzanian bean pests with this concept, we can categorise them and explain farmers' control decisions. Farmers know *O. bennigseni* in its adult form. It is easily seen, and makes an observable damage to bean leaves. The other life stages in the soil and the damage of the larvae (loss of nutrients) are difficult to see. Therefore farmers want to reduce adult abundance and leaf damage. Farmers also know bruchids in their adult form. The larva is difficult to see in the seed, and no connection between the two forms is made. They have seen emerging adults in a bean seed and therefore think that the adult beetle chews the holes into their beans. Effects of control methods can be easily seen either by comparing adult abundance or counting emergence holes/damaged beans and they therefore do not need to enquire further.

It is what farmers can easily observe that guides their explanations, but they may miss more obscure parts of the insect ecology. Farmers know aphids and the damage they are causing. They observe that ants increase when aphids are abundant and think that ants eat the aphids. As it is very difficult to see the ants "milking" the aphids, this connection is not made and no attempt to control ant abundance is undertaken. Also, farmers know the lady bird beetle and see it on plants with aphids. As it looks similar enough to *O. bennigseni*, they think that the lady bird beetle is also harmful to crops. Only showing them a beetle eating aphids convinces them otherwise. Farmers know butterflies, and they have seen their pupae, but they cannot identify it as an insect because of its immobility. They do not know the relationship between the two. They know armyworms and other lepidopteron larvae such as *Marcula testulalis* (Geyer), but they do not know that they become moths (Bottenberg, 1995). Farmers do not know the bean stem maggot or bean fly (*Ophiomyia* spp.) (Ross, 1998, Letourneau, 1994). They confuse the dark pupa in the stem of the beans with an immobile ant. They know the damage done by the pest, but attribute it to lack of water or root diseases, which often occur together with a bean fly infestation. Therefore they feel incapable of changing their fate.

(5) When farmers understand the lifecycle of the pest in more detail, they gain confidence and are more likely to teach other farmers about their control practices.

Bean fly (*Ophiomyia* spp.) is a devastating pest in beans especially under dry conditions (Abate & Ampofo, 1996). Farmers know the symptoms but not the insect causing them. They feel incapable of mitigating its effect, as they think it is related to drought, but when they irrigate, the bean roots rot and the plant often dies. For them to understand the problem and find appropriate solutions, it is necessary to learn the life cycle of the insect in the field. Bean fly was a problem in three villages, and the farmers wanted to learn solutions to control it. An approach similar to Farmer Field Schools (FFS) was used, and a small trial established to learn about this pest. At the same time this trial was used to demonstrate control options, copied partly from Ampofo & Massomo (1998b).

In one part of the field, different varieties were planted to show varietal resistance and superimposed were cultural methods using industrial fertilizer (di-ammoniumphosphate (DAP) at 200 kg/ha), DAP plus manure (at approximate 5,000 kg/ha, applied as a handfull manure into each planting hole), and DAP, manure and chemical seed dressing (Murtano® Dust: lindane 20%, thiram 26% at 3 g/kg seed). The group met every week to inspect the field and discuss expectations and check results. For instance at the first meeting just after germination of the beans, farmers expected leaves on fertilized plots to be bigger than the others, and after discussing how to be sure that this was the case, it was decided to measure the leaves (length and width) to prove it. They found that the leaves did not differ in size and the conclusion drawn was that because of the greener shade of the fertilized leaves, they seemed bigger and healthier. First punctures of oviposition by bean stem maggot were discovered and counted to see if any treatments deterred the insect from ovipositing, but no difference was detected. The next time, some plant stems were split, and larvae and pupae were shown to farmers. They also learned to recognize the tunneling of the larvae in the stem. They learned that the seed dressing kills the larvae at a very young stage. Later, plant mortality was measured and the difference between root rot and mortality due to Bean stem maggot was explained. The trial field was continually observed and yields were measured at the end of the season. Results are summarised in Fig. 1 and 2.







FIGURE 2: Yield (kg/ha) of three bean cultivars under four different treatments (DAP 200kg/ha, DAP plus manure 5,000 kg/ha, DAP plus manure plus seed dressing Murtano 3 g/kg

Farmers were very interested to learn about the cause of a problem they have experienced for years without knowing what to do about it, as they thought it to be related to their drought conditions. In a follow up session in one village, they decided that they want some written material (in Swahili) to be able to talk to their fellow farmers and help everybody to improve their bean harvests. When we showed them the material that we had put together earlier for awareness raising and education about bean stem maggot, they thought it to be very difficult to understand. We then brought them other pictures and drawings and elaborated a text that could be understood by everybody. The main changes were that some photographs were replaced by drawings and more text was used to explain local beliefs and reality about the origin of the problem. Fig. 3 shows the pictorial explanation in the two versions (English translation of the original in Swahili).



FIGURE 3: a) Picture chosen by scientists to explain the life cycle of the bean fly to farmers.b) Picture chosen by collaborating farmers to explain the life cycle of the bean fly to other farmers

In another village, where *O. bennigseni* was becoming problem, and a small group learned about the beetles life cycle in management trials; later those farmers requested local bylaws for communal action (e.g. early ploughing and crop rotation) to reduce *O. bennigseni* population, as reported in Ampofo et al. (2002). Sadly enough, the bureaucracy made it not yet happening during my time in Tanzania.

Final evaluation with collaborating and non collaborating farmers

During informal follow-up meetings about four to five years after these trials with farmers, we met with the collaborating and non-collaborating farmers to discuss what changes happened since the collaboration. Farmers continued to practice the storage methods more than other technologies. This may be partly due to what is said above about ease of observation and also partly due to saving what has been harvested as there is no compensation possible. But also the storage treatments proved to be more efficient than the treatments against O. bennigseni. Some collaborators continued to experiment on their own to adapt treatments to various pests and crops. But most not involved farmers were not aware of the trials and their outcome and did not know the new practices. This means our collaboration did not make an impact beyond the collaborating farmers. However the farmers who wrote their own extension brochure on bean fly had spread the news in the village. All farmers at the final meeting new about bean fly and *O. bennigseni*. But they spoke about a new problem in beans and that they did not harvest much. Unfortunately they did not try to investigate it or get help to find solutions. And my visit was out of season and we could not identify this new problem. I assume that they would have needed support for a longer period to become more enquiring. In conclusion, this informal evaluation confirms the results from the initial survey that complex technologies only get disseminated if a direct contact with those technologies has happened over an extended period of time (Hollenweger & Mkalimoto, 2001).

The Swahili would say: "Natuone ndipo twambe, kusikia si kuona" (let us see then we'll tell, hearing is not seeing). The farmers taking part in the trials told some farmers about their experiences, but this was not enough that they would adopt those technologies. It is imperative to include as many farmers as possible into experimentation projects, as only then will they tell others, but also those hearing will only change if they have seen. And they only see if experimentation is sustained.

There is another Swahili saying: "Nyimbo ya kufunzwa haikeshi ngoma" (learnt songs do not waken the drums (i.e. things from outside (or foreign importations) are not used for long). For a lasting change, new technologies need to be perceived as indigenous and adapted to the farmer's situation. It is important that they contributed to the outcome, because then they are their own innovation.

Already the first president of Tanzania, Mwalimu Julius Nyerere, understood this principle when he said in his book "People and Development": *"Maendeleo ni*

kuendelea kwa watu hawawezi kuendelezwa, wataweza tu kujiendeleza wenyewe inawezekani rafiki yako akakujengea nyumba, au kukulimia shamba. Lakini huwezi kujiamini na nyumba hiyo au mazao ya shamba hilo. Hutalithamini au kujivunia vitu hivyo. Vitu hivi ni lazima mtu ashirikiane na kuhusika katika ujenzi na ulimaji wake. Watu hawataendelezwa kama watafanya vitu vipya wasivyo vijua maana yake, ikiwa maendeleo ya watu ni kuwa na hali nzuri kimaisha basi hayawezi kuja kwa lazima. Usemi huu unadhibitishwa na methali isemayo "unaweza kulazimisha punda kwenda mtoni au kisimani lakini hutaweza kumlazimisha kunywa maji hayo".

(Development is the progress of people who have not been able to improve their lives. They will only be able to achieve progress by themselves: it is possible for your friend to build you a house or to farm your field, but you will not be confident in that house or the produce of that field. You will not value or pride yourself on these things. This means that it is necessary for a person to cooperate and to be concerned with their own building and farming. People will not be developed if they make new things without knowing their meaning. It is the people's development if they have a good living, but they cannot be forced to do so. This relates to a proverb which says, "you can force a donkey to go to the river or the well, but you cannot force it to drink its water" (Quoted from Marsland, 2006).

In conclusion this research shows the need to include farmers in learning trials. Only what they experience and see can be internalised to bring about change. It is crucial that farmers learn to understand life cycles of insects, or how diseases spread, so they can take simple measures to reduce their losses. The big problem is on how to free people from their busy life of subsistence to be able to learn. Farmers with smaller fields tend to prefer field days to experimental groups (Ampofo et al, 2002). Most farmer collaborators were from the middle to the higher wealth group, the others did not have the time to join in. Education on pest insects and their life cycle has to start in primary school and education needs generally become more oriented towards experiential learning and not learning by heart. Only with this understanding can the future farmers successfully take simple measures to reduce damage by pest insects.

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Penye nia ipo njia

Where there is a will, there is a way

Kujikwaa si kuanguka, bali ni kwenda mbele. To stumble is not falling down, but it is to go forward

Elimu maisha si vitabu One gets education by living, not from books alone

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1991-1992	Short training courses in preparation for working in Africa
1992	Contract work with the Agricultural Department of Zurich (5 weeks)
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1992	Development studies at the Selly Oak Colleges in Birmingham, England (11 weeks)
1993-1998	Coordinator of the Mulungwishi Agricultural Project run by the United Methodist Church in Zaire (now Democratic Republic of Congo)
1998-2004	Research fellow with the International Centre for Tropical Agriculture (CIAT) in Arusha, Tanzania
2001-2007	Doctoral studies with the Department of Agricultural Sciences and the Department of Environmental Sciences at the Swiss Federal Institute of Technology (ETH)
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