THE BEHAVIORAL ECOLOGY AND EVOLUTION OF KLEPTOPARASITISM IN AUSTRALIAN GALL THRIPS

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ABSTRACT

We used a combination of behavioral-ecological and molecular-phylogenetic data to analyze the origin and diversification of kleptoparasitic (gall-stealing) thrips in the genus *Koptothrips*, which comprises four described species that invade and breed in galls induced by species of *Oncothrips* and *Kladothrips* on Australian *Acacia*. The genus *Koptothrips* is apparently monophyletic and not closely related to its hosts. Two of the species, *K. dyskritus* and *K. flavicornis*, each appears to represent a suite of closely-related sibling species or host races. Three of the four *Koptothrips* species are facultatively kleptoparasitic, in that females can breed within damaged, open galls by enclosing themselves within cellophane-like partitions. Facultative kleptoparasitism may have served as an evolutionary bridge to the obligately kleptoparasitic habit found in *K. flavicornis*. Evidence from phylogenetics, and *Acacia* host-plant relationships of the kleptoparasites and the gall-inducers, suggests that this parasite-host system has undergone some degree of cospeciation, such that speciations of *Koptothrips* have tracked the speciations of the gall-inducers. Quantification of kleptoparasitism rates indicates that *Koptothrips* and other enemies represent extremely strong selective pressures on most species of gall-inducers. Although the defensive soldier morphs found in some gall-inducing species can successfully defend against *Koptothrips* invasion, species with soldiers are still subject to high rates of successful kleptoparasite attack. Gall-inducing thrips exhibit three main types of life-history adaptation that have apparently evolved in response to kleptoparasite pressure: (1) "fighters", which exhibit long-lived galls and soldier morphs, (2) "runners", which have quite short-lived galls, from which offspring disperse as second instar larvae, and (3) "hiders", whose galls are long-lived, especially tight-sealing, and induced on a taxonomically-distinct group of *Acacia* host plants that is seldom attacked.

Key Words: *Acacia*, cladistics, gall thrips, kleptoparasites, sociality

RESUMEN

Para analizar el origen y la diversificación de trips cleptoparásitos del género *Koptothrips,* se estudiaron aspectos sobre su comportamiento, ecología, y filogenética molecular. Este género tiene cuatro especies descritas que invaden y se crían dentro de agallas inducidas por especies de *Oncothrips* y *Kladothrips* en *Acacia* spp. australianas. El género *Koptothrips* parece ser monofilético y no estrechamente relacionado con sus hospederos. Las especies de *K. diskritus* y *K. flavicornis* parecen representar dos grupos de especies estrechamente relacionadas o de razas que difieren en sus hospederos. Tres de las cuatro especies de *Koptothrips* son cleptoparasíticas facultativas; las hembras pueden criarse dentro de agallas dañadas y abiertas protegiéndose con unos tejidos parecidos al papel celofán. El hábito de cleptoparasitismo facultativo parece haber servido como un puente evolutivo hacia el cleptoparasitismo obligado como el encontrado en *K. flavicornis*. La evidencia filogenética y de las relaciones entre la *Acacia* hospedera y los cleptoparásitos y los insectos agalleros, sugiere que este sistema de parásito-hospedero ha tenido algún grado de co-especiación ya que la espe-

ciación de *Koptothrips* ha copiado la especiación de los agalleros. La cuantificación de las proporciones de cleptoparasitismo indica que *Koptothrips* y otros enemigos naturales representan presiones selectivas sumamente fuertes sobren la mayoría de las especies de agalleros. Aunque los soldados defensivos que ocurren en algunas especies de trips agalleros pueden defender las agallas con éxito contra la invasión de *Koptothrips,* las especies con soldados de todos modos están sujetas a altas proporciones de ataque exitoso por cleptoparásitos. Los trips agalleros exhiben tres tipos principales de adaptación que han evolucionado al parecer en respuesta a la presión ejercida por los cleptoparásitos: (1) "peleadores", que producen agallas duraderas y soldados; (2) "escapadores", que producen agallas bastante efímeras y de las cuales la descendencia se dispersa durante los segundos instares larvales; y (3) "escondedores", cuyas agallas son duraderas, selladas firmemente, y que son inducidas en un grupo taxonómicamente distinto de plantas hospederas de *Acacia* que raramente son atacadas.

To survive, grow, and reproduce, all animals must engage in one or both of two strategies: utilize resources that they themselves obtain, or steal resources from others. Broadly construed, biological criminals include all predators, parasites, and parasitoids, but we usually consider parasitism as the primary example of illicit resource use. Natural selection for parasitic thievery might be expected to increase in strength with the value of the resources used, but so also would natural selection for defense. Indeed, defense against parasites has been considered the main selective pressure for much of the spectacular, beautiful, and stunningly-complex diversity of adaptation, including sex (Hamilton et al. 1990), sexual selection (Hamilton and Zuk 1982; Andersson 1994), social cooperation (Lin 1964; Lin and Michener 1972; Alexander 1986), immune systems, and even multicellularity itself (Frank 1994). How the evolutionary dynamics of host-parasite attack and defense play out for any set of species depends on the variation available for selection, the genetic bases of the variation, and the degree to which each party can control resource use. As such, in-depth analyses of the ecology and evolution of particular host-parasite systems are necessary to uncover the general principles underlying the forms and maintenance of these two strategies.

The purpose of this paper is to describe and test hypotheses for the origin, evolution, and behavioral ecology of kleptoparasites in Australian gall thrips, especially with reference to the evolution of various forms of defense against these enemies. Kleptoparasites are a special subset of natural enemies that usurp valuable physical resources from the creator or obtainer, and thus fit most closely with our human perspective on theft. We first describe the natural history and ecology of kleptoparasitic thrips and their victims, thrips that induce galls on species of Australian *Acacia*. Second, we present evidence from phylogenetics designed to uncover the evolutionary origins of the kleptoparasitic lifestyle, and the patterns in its diversification. Finally, we present data from behavioral-ecological studies that are focused on understanding the ecology and evolution of these host-kleptoparasite interactions, and we fit these data on processes into our phylogenetic framework.

Materials and Methods

Natural history of Australian gall-inducing thrips and their kleptoparasites

A total of 21 described species of gall-inducing thrips in the genera *Kladothrips*, *Oncothrips*, and *Onychothrips* induce galls on phyllodes (petioles modified to function as leaves) of *Acacia* in the sections Plurinerves, Juliflorae, and Phyllodinae (Mound et al. 1996). Three described species, *K. rugosus*, *O. habrus*, and *O. waterhousei*, apparently

each represents a suite of host-specific sibling species, inducing galls on different species of plant (Crespi et al. 1998). Galls are induced on young, actively-growing phyllodes by single adult macropterous females, and in some *Kladothrips* species a male sometimes joins a female during gall initiation. During the period from gall initiation until closure, females of *Oncothrips tepperi, O. habrus, Kladothrips rugosus, Onychothrips arotrum* and *Ony. tepperi* have been observed to fight with one another over gall ownership, using their enlarged, armed forelegs (Crespi 1992a,b, Mound and Crespi 1995). All other known gall-forming species, except *Oncothrips rodwayi* and *O. antennatus*, have notably enlarged forelegs in the females which are also indicative of fighting. Males are also known to fight one another during gall induction in *K. rugosus*, and their enlarged forelegs suggest that males also fight in *K. ellobus* and *K. acaciae*.

After successfully inducing a gall, the enclosed female lays eggs on the inner surface of the gall, which develop into larvae that feed within the gall. Three main forms of life-history are exhibited in the gall-inducers. First, in some species (*Kladothrips rugosus, K. acaciae*, and *K. ellobus*), the length of time spent in the gall by developing larvae is short relative to other gall-inducers on *Acacia*, on the order of 1-2 months, and mature second-instar (i. e., last-instar) larvae apparently leave the gall prior to pupation in the soil (B. Crespi and B. Kranz, personal observation). Second, in some species (*Oncothrips tepperi, O. habrus, O. waterhousei, O. morrisi, K. hamiltoni*, and *K. harpophyllae*), some or all of the offspring produced by the foundress develop into wing-reduced or wingless "soldier" morphs, which have enlarged forelegs that they use to defend the gall against interspecific invaders (Crespi 1992b; Mound and Crespi 1995; Crespi et al. 1997; Crespi and Mound 1997). Third, some species (*Oncothrips antennatus, O. schwarzi, Onychothrips arotrum, Ony. tepperi, Ony. zygus* and *K. auggonsaxxos*) do not exhibit soldier morphs, but the offspring of the foundress all eclose within the gall. A variant of this life cycle is exhibited by *Oncothrips sterni*, in which a cohort of wingless, larviform, non-soldier adults develop within the gall, and apparently breed and contribute to the production of winged dispersers (Mound et al. 1996, Crespi and Mound 1997).

The genus *Koptothrips* comprises four described species, *K. xenus, K. zelus, K. dyskritus,* and *K. flavicornis* (Mound 1971, Crespi 1992a, Crespi and Mound 1997), all of which kleptoparasitize galls of the gall-inducers. *K. xenus* and *K. zelus* are host-specific, attacking *Kladothrips ellobus* and *K. acaciae* respectively, whereas specimens that key to *K. dyskritus* attack the various sibling species of *K. rugosu*s (and possibly other taxa), and specimens that key to *K. flavicornis* attack *O. tepperi, O. habrus, O. waterhousei, K. rugosus*, and several other species. *Koptothrips* are found in galls of all stages, from recently-founded by the gall-inducer to at least several months old. Galls are usually invaded by single females, which attack and attempt to kill the gall-forming thrips inside. If successful, a female *Koptothrips* produces a single brood of female and male offspring, which disperse from the gall as adults. In *K. zelus* and *K. xenus*, multiple females may invade a gall, in which case the females are each found in a small section of the gall which is partitioned from other sections by a cellophane-like material, apparently produced by the thrips (Crespi and Mound 1997). Females of *K. dyskritus* have also been collected from within cellophane-like partitions that they create with accessory gland secretions to enclose them within sections of damaged, otherwise-open galls of *K. rugosus* that no longer contain any of the gall-inducers (Crespi, unpublished data; see also Mound et al. 1997).

Encounters between females of *K. flavicornis* and *K. dyskritus,* and adults of various species of *Oncothrips* and *Kladothrips,* have been observed in galls in the laboratory (Crespi 1992b, Crespi and Mound 1997). Winged foundresses of the gall-inducers, and soldier morphs in the *Oncothrips* and *Kladothrips* with soldiers, will fight the *Koptothrips*, attempting to grasp and kill them with their enlarged forelegs, which are armed

at the apex with sharp, pointed fore-tarsal teeth. The *Koptothrips* usually fight back, also by grasping and stabbing with their forelegs and fore-tarsal teeth, and if they successfully pierce a gall-inducer with their fore-tarsal teeth, the pierced individual usually dies within a few minutes (Crespi and Mound 1997). Galls successfully invaded by any of the four *Koptothrips* taxa always contain a dead foundress or a dead foundress and founder male, which indicates that invading *Koptothrips* kill the gall-inducers.

Molecular-phylogenetic analysis

The first main goal of our phylogenetic analysis was to assess the taxonomic status of *Koptothrips flavicornis* and *K. dyskritus* collected from galls of host thrips species on different host plants. Each of these two described species may represent either a single, more or less panmictic, generalist species, or a suite of more or less specialized species, each attacking a different species of gall-inducing thrips. For *K. flavicornis*, Mound (1971) noted that specimens collected from the galls of different host thrips often vary considerably in color, ranging from bicolored with a reddish head and yellow abdomen, to brownish, to black. Although mitochondrial DNA sequence data cannot unambiguously indicate species status, levels of divergence between putative taxa can help in achieving this goal (Avise 1994).

The second main goal of our phylogenetic analysis was to evaluate the evolutionary relationships between the *Koptothrips* and their hosts. Alternative hypotheses for these relationships, all of which have been discussed with reference to other taxa (e. g. Bourke and Franks 1991, Choudhary et al. 1994, Ward 1996, Lowe and Crozier 1997, Morris et al. 1998) include: (a) monophyly of the kleptoparasites, and of the hosts, and a lack of sister-taxon status between the two; (b) monophyly, and sister-taxon status, of the kleptoparasites and hosts; (c) monophyly of the kleptoparasites, but paraphyly of the hosts with respect to the kleptoparasites, such that the parasites evolved from within the host lineage; or (d) sister-taxon relationships between each or most of the pairs of hosts and parasites, such that neither is monophyletic. As discussed below, these hypotheses imply different sets of ecological and behavioral mechanisms for the origin and diversification of the hosts and kleptoparasites.

To infer a phylogeny for the gall-inducing thrips, we used a combination of data from mitochondrial DNA sequence from the COI and 16S genes, adult morphological characters, and gall morphology characters (Crespi et al. 1998). For the kleptoparasites, we used about 450 base pairs of mitochondrial DNA sequence from the COI gene for all taxa, with about 250 base pairs of 16S for some taxa. Sequence data for most of the gall-inducers included here is given in Crespi et al. (1998), and all other data (e. g., for all of the *Koptothrips*) is described and analyzed below. Procedures used for DNA isolation, PCR, and sequencing, and sequence for the gall-inducing taxa, are described in Crespi et al. (1998). As described below, we used *Gynaikothrips ficorum* as our outgroup (Crespi et al. 1998), and maximum parsimony analysis and neighbor joining in PAUP 4.0 (Swofford 1998) to analyze the data. We used 500 bootstrap replicates with neighbor joining to assess the robustness of the neighbor joining tree; maximum parsimony bootstrapping was computationally impossible due to the large number of taxa in our data set.

Measurement of kleptoparasitism rates

We collected data on rates of successful kleptoparasitism in the field to evaluate the prevalence and patterns of kleptoparasitism in different host species. Galls were collected from numerous sites throughout Australia (Table 1) directly into 60-100%

ethanol. Any given site includes galls from one to several dozen *Acacia* trees, and we either collected all galls encountered (when galls were rare), or collected so as to obtain a representative sample of galls with respect to variation in size. For all species, hosts or kleptoparasites in galls from a given site are quite synchronized in their life cycle, as a result of breeding cued by either an annual cycle of new shoot growth, or rainfall.

We dissected galls in the laboratory and recorded the species of thrips inside, and their life-cycle stages present, as well as the presence of other invaders. The predominant non-thysanopteran invaders were lepidopteran larvae (mainly or all species of Lepidoptera in the family Cosmoptyrigidae, one to a gall), which eat gall tissue from the inside and lead to a drastic reduction in thrips numbers, but usually do not kill all of the gall inhabitants. In some galls, both *Koptothrips* and a lepidopteran larva were present, but *Koptothrips* and gall-forming thrips never coexisted alive, except in several galls of *Oncothrips antennatus* on *Acacia adsurgens*. We calculated percent *Koptothrips* invasion as the number of galls containing live *Koptothrips* divided by total gall number, and made similar calculations for non-thysanopteran invaders (lepidopteran and dipteran larvae). Thus, the kleptoparasitism data refer to rates of suc-

TABLE 1. RATES OF SUCCESSFUL *KOPTOTHRIPS* KLEPTOPARASITISM, AND PARASITISM BY LEPIDOPTERANS AND DIPTERANS ("OTHER INVADERS"), GIVEN AS MEANS AND STANDARD DEVIATIONS (AVERAGING ACROSS COLLECTIONS). * = GALL-INDUCING THRIPS SPECIES WITH SOLDIER MORPHS.

Gall-inducing thrips species	Number of collections	Number of galls	% *Koptothrips* invaders	% Other invaders
*Oncothrips morrisi	1	24	0.29	0
*Oncothrips waterhousei	2	68	0.32 (0.003)	0.44 (0.09)
*Oncothrips habrus1	5	240	0.23 (0.18)	0.17 (0.07)
*Oncothrips habrus2	2	38	0.08 (0.002)	0.51 (0.37)
*Oncothrips tepperi	10	423	0.31 (0.26)	0.29 (0.23)
Oncothrips rodwayi	3	125	0.07 (0.02)	0.30 (0.24)
*Kladothrips hamiltoni	6	215	0.25 (0.15)	0.36 (0.17)
*Kladothrips harpophyllae	1	20	0.25	0.35
Kladothrips rugosus1	6	237	0.19 (0.15)	0.24 (0.19)
Kladothrips rugosus2	6	171	0.04 (0.04)	0.03 (0.04)
Kladothrips rugosus3	4	133	0.03 (0.06)	0.11 (0.19)
Kladothrips ellobus	6	147	0.40 (0.26)	0.16 (0.21)
Kladothrips acaciae	4	84	0.35 (0.30)	0.03 (0.06)
Oncothrips antennatus1	3	105	0.24 (0.04)	0.19 (0.01)
Oncothrips antennatus2	10	315	0.02 (0.05)	0.45 (0.25)
Onychothrips arotrum	6	229	0	0.16 (0.13)
Onychothrips tepperi	1	11	0	0

*Oncothrips habrus*1 = *O. habrus* on *Acacia melvillei*; *O. habrus*2 = on *A. pendula*; *K. rugosus*1 = on *A. pendula*; *K. rugosus*2 = on *A. melvillei*; *K. rugosus*3 = on *A. tephrina*; *O. antennatus*1 = on *A. adsurgens*; *O. antennatus*2 = on *A. aneura*.

cessful attack and invasion. We have no information concerning what proportion of attacks by *Koptothrips* is unsuccessful, except to note that dead *Koptothrips* adults are occasionally found within galls containing live gall-forming thrips (Crespi 1992a, Mound and Crespi 1995). Many attacks could be aborted, however, before the invader fully enters the gall.

RESULTS

Molecular Phylogenetics

The percent sequence divergence in the COI gene between *K. flavicornis* collected from different host thrips taxa and *Acacia* species ranged from 0 to 5.6% (with most of the values between 2.5 and 5.6%), and divergences for *K. dyskritus* ranged from 0.5 to 6.9% (with one value of 0.5%, and five values between 5.5% and 6.9%). For *K. flavicornis*, divergences were especially low between specimens collected from two different host thrips species on the same host plant (0.2% for specimens collected from *K. rugosus* and *O. waterhousei* on *A. loderi*, and 0.2% for specimens collected from *K. rugosus* and *O. waterhousei* on *A. ammophila*).

Maximum parsimony analysis of the mitochondrial DNA and morphology data yielded six shortest trees of length 1484. The strict consensus of these trees was well resolved (Fig. 1a) and indicated that the gall-inducing thrips, and the *Koptothrips*, each forms a monophyletic group. Within the *Koptothrips*, each described species was also monophyletic, with *K. xenus* and *K. zelus* most basal, *K. zelus* forming the sister-group to *K. dyskritus*, and (*K. zelus* + *K. dsykritus*) as sister-group to *K. flavicornis*.

Neighbor-joining analysis of the COI and 16S mitochondrial DNA data yielded a tree that was closely-similar to the maximum parsimony tree, the main differences being the monophyletic status of (*Koptothrips dyskritus* + *K. xenus* + *K. zelus*), and sister-taxon status of *Koptothrips xenus* and *K. zelus,* in the neighbor joining tree (Fig. 1b). About half of the nodes in the neighbor-joining bootstrap tree were well-supported by the bootstrap (with support of 70% or higher) (Fig. 1c). In particular, the bootstrap provided strong support for monophyly of the gall-inducers, and monophyly of the kleptoparasites, but it provided relatively weak support for the placements of *K. zelus* and *K. xenus*.

Consideration of the host plants inhabited by the gall-inducing thrips and their kleptoparasites, with respect to their positions in the maximum-parsimony phylogeny, reveals a notable pattern: the relatively basal kleptoparasite species *K. xenus* and *K. zelus* attack the relatively-basal host species *Kladothrips acaciae* and *K. ellobus* respectively. Moreover, all four of these species are host-insect and host-plant specific, and all are quite morphologically distinct from their closest relatives (Mound 1971). This finding suggests that, as described in detail below, the diversification of *Koptothrips* and their gall-inducing hosts has involved some degree of cospeciation. Further evidence for cospeciation is provided by two additional patterns. First, *K. dyskritus*, which apparently descended from the ancestor of (*K. xenus* + *K. zelus*), attacks primarily *K. rugosus*, which apparently descended from the ancestor of *K. ellobus* and *K. acaciae*. Second, *K. flavicornis*, which is sister-taxon to the other *Koptothrips* taxa, mainly attacks gall-inducing thrips in the (*Oncothrips morrisi* + *O. waterhousei* + *O. habrus* + *O. tepperi* + *O. rodwayi*) clade, which forms the sister-group to the *Kladothrips* species.

The two *Koptothrips* taxa that appear derived with respect to *K. xenus* and *K. zelus*, *K. dyskritus* and *K. flavicornis*, each attacks multiple species of gall-inducing thrips, and the gall-inducing thrips that they attack, primarily *K. rugosus*, *O. waterhousei*, and *O. habrus*, also each appears to represent a set of closely-related species

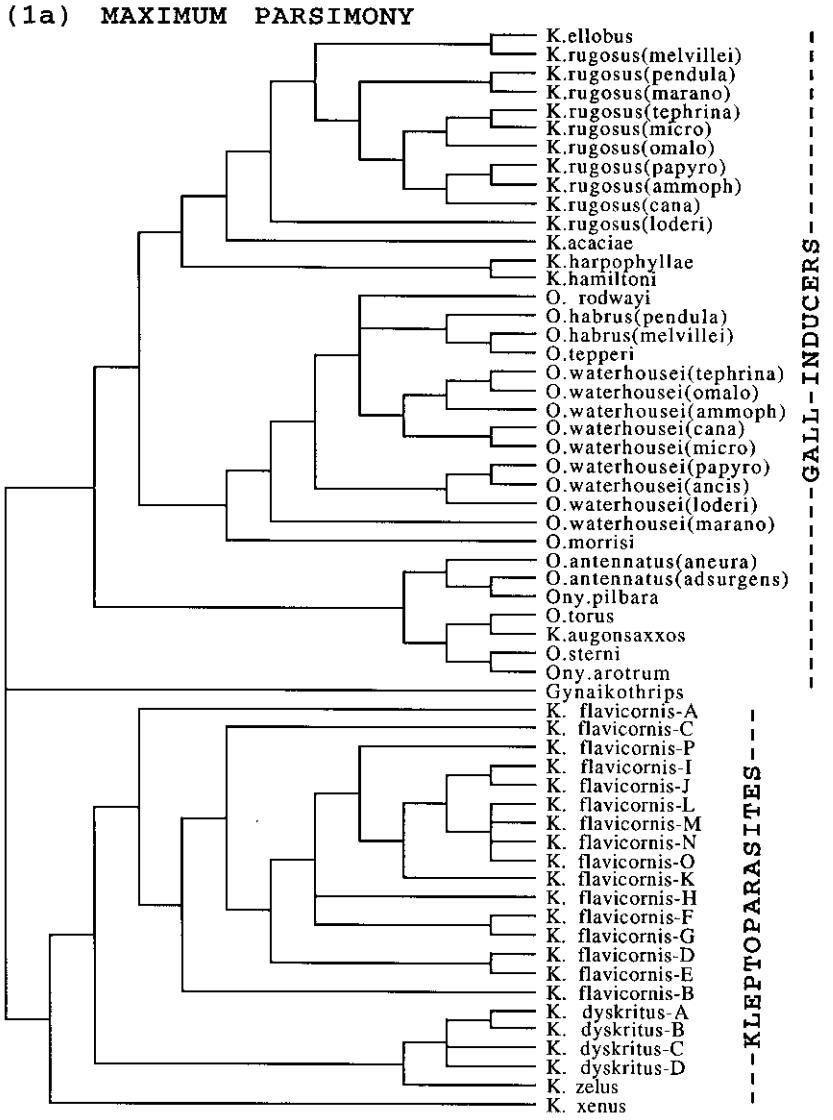

Fig. 1. (a) Strict consensus of six most-parsimonious trees of length 1484, inferred using heuristic searching in PAUP 4.0. For the gall-inducing species comprising sibling species or host races on different host plants, the thrips species name is followed by a code for the species name of the *Acacia*. For the *Koptothrips*, specimens of *K. flavicornis* and *K. dyskritus* collected from galls from different host-thrips species are given unique letter codes. Complete host localities and other collection information is available from BJC.

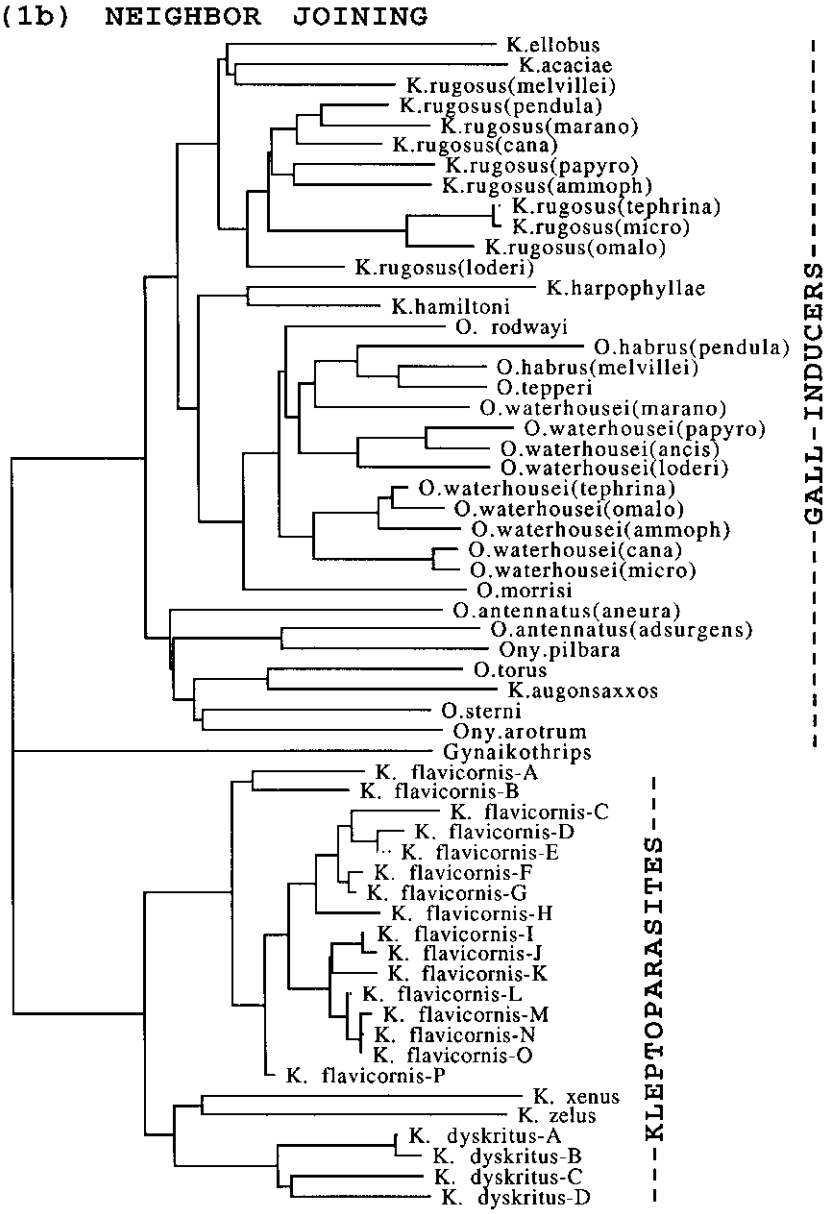

Fig. 1. (b) Neighbor-joining tree, inferred from the mitochondrial COI and 16S DNA data. For the gall-inducing species comprising sibling species or host races on different host plants, the thrips species name is followed by a code for the species name of the *Acacia*. For the *Koptothrips*, specimens of *K. flavicornis* and *K. dyskritus* collected from galls from different host-thrips species are given unique letter codes. Complete host localities and other collection information is available from BJC.

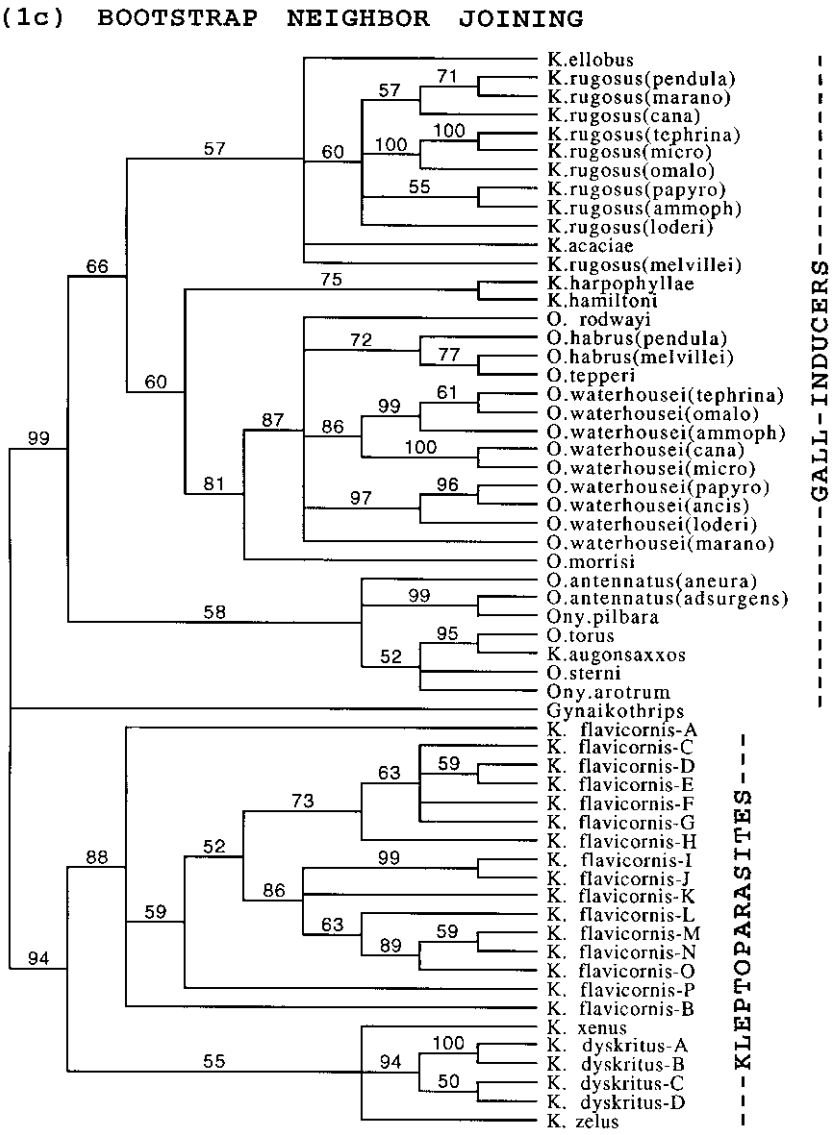

Fig. 1. (c) Neighbor-joining bootstrap tree (500 replicates). For the gall-inducing species comprising sibling species or host races on different host plants, the thrips species name is followed by a code for the species name of the *Acacia*. For the *Koptothrips*, specimens of *K. flavicornis* and *K. dyskritus* collected from galls from different host-thrips species are given unique letter codes. Complete host localities and other collection information is available from BJC.

on different *Acacia* host-plants (Crespi et al. 1998). However, whereas *K. dyskritus* primarily attack species of *Kladothrips rugosus*, *K. flavicornis* commonly attack *O. waterhousei* and *O. habrus*, *O. tepperi*, *O. rodwayi*, and sometimes *K. rugosus*. These data suggest that the diversification of *Koptothrips* has involved an expansion of host range if *K. flavicornis* represents a single species, or a radiation involving diverse hosts if it represents a suite of closely-related sibling species.

Mapping of the behavior of *Koptothrips* onto the maximum-parsimony phylogeny reveals another notable result: *Koptothrips xenus,* which is one of the three *Koptothrips* taxa known to be facultatively kleptoparasitic, is basal with respect to the lineage giving rise to the obligately-kleptoparasitic taxon *K. flavicornis.* Thus, facultative kleptoparasitism is inferred as ancestral for the genus *Koptothrips*, with one inferred shift to obligate kleptoparasitism. This finding supports the hypothesis that kleptoparasitism in *Koptothrips* originated as a facultative alternative, and became obligate in association with the speciation event that gave rise to *K. flavicornis*.

In our neighbor-joining tree, the three facultatively-kleptoparasitic *Koptothrips* taxa form a monophyletic group, which is sister-taxon to *K. flavicornis*, and (*K. xenus* + *K. zelus*) also forms a monophyletic group. This phylogeny is also broadly compatible with a cospeciation model, in that the two main lineages of hosts, *Oncothrips* and *Kladothrips*, are attacked respectively by the two main lineages of kleptoparasites, *K. flavicornis* and (*K. dyskritus* + *K. zelus* + *K. xenus*), and the *K. zelus* and *K. xenus* lineages appear old and divergent relative to the other two *Koptothrips* species. The weakness of the bootstrap support for the positions of *K. xenus* and *K. zelus* in this tree indicates that we cannot consider the results of the maximum-parsimony and neighbor-joining analysis incompatible. These analyses also tell us that additional data from a more slowly-evolving molecule would help to resolve the positions of these two species.

Kleptoparasitism Rates

Data on rates of successful kleptoparasitism, and successful invasion by lepidopterans and dipterans, are summarized in Table 1. Four patterns are notable in these data. First, the highest kleptoparasitism rates are found in the two species, *Kladothrips acaciae* and *K. ellobus*, that are attacked by the host-specific, morphologically-specialized invaders *Koptothrips zelus* and *K. xenus*. Second, rates of kleptoparasitism are also quite high in five of the six species of gall-inducing thrips with soldier castes, with on the order of one-quarter to one-third of galls successfully invaded. Indeed, rates of *Koptothrips* invasion are about twice as high overall in species with soldiers as in species without soldiers. Third, some of the lowest rates of kleptoparasitism are exhibited by three species, *Onychothrips arotrum*, *Onychothrips tepperi*, and *Oncothrips antennatus* on *Acacia aneura*, that are related phylogenetically, all being found in the same monophyletic group. Moreover, this clade is unusual in that all of its species induce galls on *Acacia* species in the section Juliflorae, whereas all of the other species induce galls on *Acacia* in the section Plurinerves. Finally, rates of invasion by non-thysanopterans, mainly lepidopterans and dipterans, do not show the same clear interspecific patterns as those for kleptoparasites; instead, almost all of the gall-inducers are heavily beset by these enemies.

DISCUSSION

The main goal of this study is to understand the evolutionary and behavioral-ecological dynamics of the kleptoparasite-host relationships found in *Koptothrips* and gall-inducing thrips on Australian *Acacia*. To achieve this goal, we have (1) used mi-

tochondrial DNA data to assess the taxonomic status of two taxa, *Koptothrips flavicornis* and *K. dyskritus*, that attack multiple species of gall-inducing thrips on multiple host plant species; (2) tested hypotheses for the evolutionary origin of kleptoparasitism in these insects, (3) analyzed the phylogenetic and behavioral-ecological patterns of diversification of *Koptothrips*, with respect to the diversification of their hosts, and (4) used data on rates of kleptoparasitism in different species to draw inferences concerning its importance as a selective pressure. Our hypothesis for the origin and diversification of *Koptothrips* and their hosts is depicted and summarized in Fig. 2, and described in detail below.

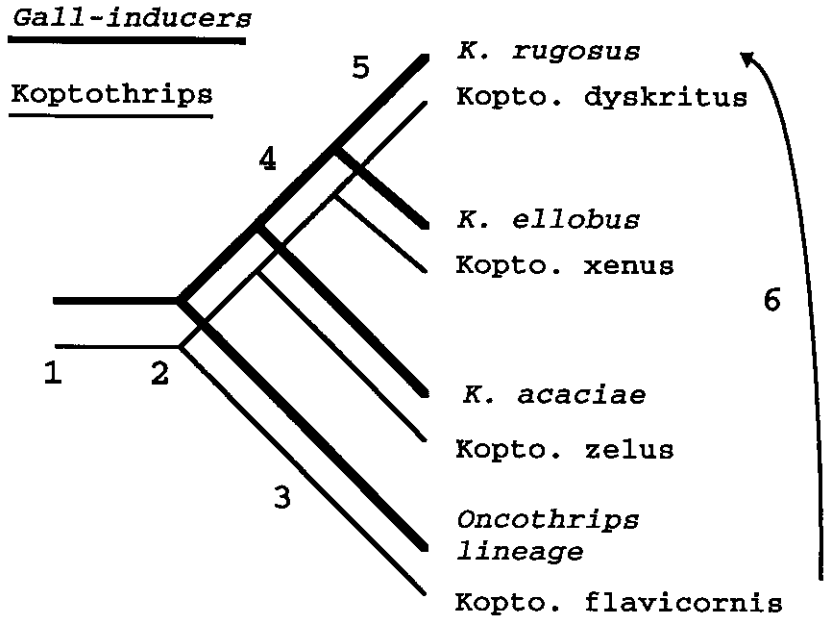

Fig. 2. Hypothesized scenario for the broad-scale evolutionary relationships between Australian gall-inducing thrips on *Acacia* and their *Koptothrips* kleptoparasites. (1) Origin of the genus *Koptothrips*, via a host shift onto *Acacia*, attacking the ancestor of the (*Oncothrips* + *Kladothrips)* lineage of gall-inducers. (2) Ancestor of (*Oncothrips* + *Kladothrips*) splits into two genera, and, as a result, *Koptothrips* splits into two lineages, one giving rise to (*K. xenus* + *K. zelus* + *K. dyskritus*) (which attack *Kladothrips*), the other giving rise to *K. flavicornis* (which attack *Oncothrips*). (3) *Oncothrips* lineage and *Koptothrips flavicornis* lineage diversify. (4) *Kladothrips acaciae* and *K. ellobus* descend from the ancestral *Kladothrips* lineage, leading to the evolution of their host-specific *Koptothrips*, *K. zelus* and *K. xenus*. (5) Next, *Kladothrips rugosus* originates along the *Kladothrips* lineage, leading to the evolution of *Koptothrips dyskritus*. *K. rugosus* and *K. dyskritus* diversify together, onto different species of *Acacia* in the section Plurinerves. (6) Some *Koptothrips flavicornis* lineages expand their host range by attacking *Kladothrips rugosus* that are on the same host plant as their ancestral *Oncothrips* hosts. By plausible alternative phylogenies, *Kladothrips acaciae* and *K. ellobus* may be sister-taxa, and *Koptothrips zelus* and *K. xenus* may also be sister taxa. We stress that this diagram represents a hypothesis that, although consistent with our available data, requires additional testing.

The levels of mitochondrial DNA sequence divergence found between specimens of *K. flavicornis* and *K. dyskritus* collected from different species of host thrips on different *Acacia* species range up to 7% and average about 3%. These values are consistent with the hypothesis that each of these named species actually comprises a set of multiple closely-related sibling species or host races. However, we also note that some of the pairwise divergences within these taxa are very low, below 0.5%, and that two of these low divergence values are for samples of *K. flavicornis* collected from different species of host thrips (*K. rugosus* and *O. waterhousei*) each on the same host plant (*A. loderi* or *A. ammophila*). These findings strongly suggest that whereas some *K. flavicornis* are sufficiently genetically divergent from others that high levels of gene flow are unlikely to be occurring between them, the *K. flavicornis* attacking different host thrips species on the same host plant may well be conspecific. Further analysis of the systematic status of *K. flavicornis* and *K. dyskritus* requires quantification of genetic variation both between and within putative conspecific populations, and experimental transfer of *Koptothrips* between host thrips and host plants.

Our phylogenetic analyses indicate that the genus *Koptothrips*, and its gall-inducing host species, are each monophyletic. These results falsify the hypothesis that *Koptothrips* arose from within the lineage of gall-inducers on *Acacia*, ostensibly via intraspecific kleptoparasitism during gall induction (Crespi 1992a). Because our phylogeny does not yet include genera of thrips on Australian *Acacia* other than *Oncothrips*, *Kladothrips*, *Onychothrips*, and *Koptothrips* (Mound 1971), we must turn to other information to assess whether or not *Koptothrips* and their gall-inducing hosts are (1) sister-taxa, such that they share a common ancestor, or (2) not closely related, such that kleptoparasitism arose via a host-plant shift. Using a cladistic morphological analysis of Australian Phlaeothripines, Morris et al. (1998) have shown that the latter hypothesis is supported. By their analysis, the genus *Koptothrips* is not closely-related to its gall-inducing hosts, nor is it found in a clade of thrips that inhabits *Acacia*; instead, it appears to be related to species of *Teuchothrips*, which induce simple leaf-roll or curl galls on a variety of plant taxa. Since *Koptothrips* are not known to attack *Teuchothrips*, these results suggest that the genus *Koptothrips* originated, and evolved its kleptoparasitic habit, in conjunction with a major host-plant shift onto *Acacia* (Morris et al. 1998). This hypothesized scenario for the origin of *Koptothrips* is depicted as stage 1 in Fig. 2. Whether the progenitors of *Koptothrips* were gall-inducers like *Teuchothrips*, or non-galling plant feeders, cannot be inferred from the phylogenetic information available to date.

What might be the ecological basis and evolutionary significance of kleptoparasitism originating in association with a host-plant shift? Morris et al. (1998) suggest that the habit of invading galls could have facilitated host-shifting because galls provide a highly favorable microhabitat, especially in a climate like that of arid-zone Australia. Thus, the advantages of using galls as domiciles could have helped offset the disadvantages of adapting to live on a novel host plant. Moreover, in the same way that host-plant shifts by phytophagous insects can be facilitated by escape from natural enemies (Brown et al. 1995, Feder 1995, Shorthouse and Brooks 1998), we suggest that host-insect shifts by incipient enemies could be facilitated by a lack of evolved defenses of their hosts. Support for a hypothesis of host-shifting coinciding with the origin of kleptoparasitism comes from remarkably parallel situations in two taxa unrelated to gall thrips: (1) in *Eriosoma* aphids, kleptoparasitism of galls has also apparently originated via a host-plant shift (Akimoto 1981, 1989), and (2) in yucca moths, phylogenetic evidence indicates that non-pollinating 'cheater' species originated in association with host-plant shifts (Pellmyr et al. 1996). Our hypothesis of evolutionary cheating arising as a result of ecological-phylogenetic saltation could be tested further by designing phylogenetic studies to uncover the ecological habits of

the closest honest relatives of such cheaters, rather than focussing just on the parasites and hosts when analyzing the origin of the parasites.

Phylogenetic and behavioral data suggests that, in addition to involving a host-plant shift, the origin of kleptoparasitism in *Koptothrips* may also have involved a facultative stage. This hypothesis is supported by the observation that *K. xenus*, *K. zelus* and *K. dyskritus* can create cellophane-like partitions in damaged, open galls bereft of gall-inducers, and breed successfully inside. By contrast, the obligately-kleptoparasitic *K. flavicornis* apparently cannot do so. We suggest that the primordial *Koptothrips* used damaged, open galls for breeding, as do some *Grypothrip*s and *Katothrips* (Crespi et al. 1997), that facultative kleptoparasitism evolved via selection for obtaining a better, larger, and fresher resource for breeding, and that obligate kleptoparasitism evolved in the *K. flavicornis* lineage via evolutionary refinement of usurpation behavior (see Field 1992 for description of similar patterns in some Hymenoptera). This hypothesis fits with West-Eberhard's (1986) model of evolutionary transitions arising from facultative alternative behaviors, and it could be tested via more-detailed study of *Koptothrips* morphology and behavior in the context of their phylogeny. In particular, we need better resolution and support for the phylogenetic placements of *Koptothrips xenus* and *K. zelus,* for which neighbor-joining and maximum-parsimony yield differing, albeit weakly-supported, results.

Once a parasitic habit has evolved, diversification of parasite lineages can proceed via two main mechanisms: cospeciation, such that the parasites simply track the speciations of their hosts (Page 1994), and host-shifting, such that parasites move between host species more or less respective of the phylogenetic affinities of their current and future hosts. Our phylogenetic data indicates that the oldest split in the gall-inducers on *Acacia* in the section Plurinerves was between the genera *Oncothrips* and *Kladothrips*, and it suggests that this split was mirrored by *Koptothrips* kleptoparasites, as they diversified into two clades, *K. flavicornis*, which attack mainly *Oncothrips*, and (*K. zelus* + *K. xenus* + *K. dyskritus*), which attack species of *Kladothrips* (stage 2 in Fig. 2). Moreover, within the *Kladothrips* lineage, *K. acaciae* and *K. ellobus* are the most-basal species, and they are attacked by the two *Koptothrips* species that appear relatively old and basal, *K. zelus* and *K. xenus*. These relationships are also supported by the similarity in levels of divergence in mtDNA between *K. xenus* and *K. zelus* (14%), and between *K. ellobus* and *K. acaciae* (13.3%), which suggests that the two pairs of lineages may be of similar ages.

What of *K. dyskritus* and *K. flavicornis*? *K. flavicornis*, which our phylogeny identifies as the sister-taxon to the other *Koptothrips* species, attack species of gall-inducers in the sister-taxon of (*K. acaciae* + *K. ellobus* + *K. rugosus)*, which comprises species of *Oncothrips*. This pattern suggests that the ancestor of *K. flavicornis* attacked the ancestor of (*O. morrisi* + *O. waterhousei* + *O. habrus* + *O. rodwayi* + *O. tepperi*) (stage 3 in Fig. 2), and has diversified by some combination of cospeciation, host shifting, and perhaps independent speciation. The descent of *K. dyskritus* from the ancestors of *K. xenus* and *K. zelus* is compatible with the observation that *K. dyskritus* primarily attack *Kladothrips rugosus*, a lineage that has descended from the ancestor of *K. acaciae* and *K. ellobus* (stages 4 and 5 in Fig. 2). Thus, our phylogeny is consistent with the hypothesis that cospeciation was also involved in the evolution of *K. dyskritus*. Finally, the observation that some *K. flavicornis* attack *K. rugosus*, as well as species of *Oncothrips*, suggests that some *K. flavicornis* lineages have undergone a host-insect range expansion, to include *K. rugosus* in their list of victims (stage 6 in Fig. 2). Our data also indicate that the *Acacia* host plants have mediated the putative expansion of host range: in both cases where *K. flavicornis* attack both *Oncothrips* and *Kladothrips*, the two species of gall-inducers are on the same host-plant species.

Further analyses of the evolution of across trophic-level interactions in *Koptothrips* and their hosts requires: (1) more thorough sampling of the *Koptothrips* from different host thrips and host plants, (2) more detailed elucidation of the taxonomic status of the *K. flavicornis* and *K. dyskritus* attacking different hosts, (3) statistical analysis of cospeciation models (Page 1994), (4) better understanding of the mechanisms responsible for cospeciation and host-shifting, (5) better support for the phylogenetic positions of *Koptothrips zelus* and *K. xenus*. However, the data presented here suggest that *Koptothrips* and their hosts have evolved together via some combination of cospeciation and host shifting, which will make them especially useful for analyzing the causes of these disparate processes.

Within lineages, kleptoparasitism evolves as a consequence of natural selection on both the parasites and their hosts, and our data on rates of kleptoparasitism allows us to assess its importance as a selective pressure in host species that differ in various aspects of their life history. Our quantification of rates of parasitism by *Koptothrips* and non-thysanopterans has uncovered four main patterns.

First, the highest rates of *Koptothrips* kleptoparasitism occur in *Kladothrips ellobus* and *K. acaciae*, the only two species with host-specific kleptoparasites, *Koptothrips xenus* and *K. zelus*. These high kleptoparasitism rates may be due in part to highly-developed parasite specialization, if higher kleptoparasite efficiency has evolved as a consequence of adaptation to single rather than multiple hosts (see Bernays and Graham 1988). Similarly high rates of successful attacks by specialist enemies have been reported in a subsocial pentatomid bug (Eberhard 1975), and in some species of gall aphids (e. g., Itô 1989, Moffett 1989, Stern and Foster 1996).

Second, rates of successful *Koptothrips* parasitism are also quite high in almost all species with soldiers. At first glance, this result might appear paradoxical, because behavioral evidence from numerous species with soldiers indicates that soldiers fight, and often kill, invading *Koptothrips* (Crespi and Mound 1997, Crespi unpublished data). However, such defense, though often spectacular, is frequently impossible or ineffectual: *Koptothrips* sometimes invade galls before any soldiers have eclosed (Crespi and Mound 1997), and *Koptothrips* are often victorious in their fights with soldiers. Moreover, if high rates of kleptoparasitism were important in selecting for the origin of soldier castes (Crespi 1996, Crespi et al. 1998), then such high rates should not surprisingly be instrumental in maintaining soldiers.

The hypothesis that parasite pressure has been an important cause of the origin and maintenance of sociality could be analyzed further by comparing parasite and predator pressure between non-thysanopteran taxa with and without soldiers or other types of worker that defend. At present, such data are available for only two taxa (see also Crespi and Choe 1997). Moran (1993) compared rates of successful predation by a gall-specializing fly larva between a *Pemphigus* gall aphid species with soldiers, and a *Pemphigus* species without soldiers, for galls taken from the same tree. The aphid species with soldiers had lower predation rates than the species without soldiers for most of the season, but rates of unsuccessful attack on the species with soldiers are unknown. Schwarz (1994) found higher rates of parasitism by encyrtid wasps and cuckoo bees, and higher levels of cooperative nests and nest aggregation, in montane populations of the allodapine bee *Exoneura robusta* (= *bicolor*) than in heathland populations of the closely related species *Exoneura nigrescens*. Rates of parasitism by encyrids did not covary with colony site at either location, but higher numbers of females may help in defense against cuckoo bees. By contrast, larger colony sizes have been inferred to enhance protection of brood against ants in *Allodapula melanopus* (Michener 1971), in *Exoneura robusta* (Schwarz 1986, 1994) and in *Exoneura nigrescens* (Bull and Schwarz 1996). Among allodapines in general, species exhibiting eusociality ap-

pear more beset by inquiline bees than are species with only solitary nests, but whether high inquilinism rates are a cause or effect of sociality is not yet known.

Third, some species of gall-inducing thrips, notably some *K. rugosus, O. antennatus* from *Acacia aneura*, *Onychothrips arotrum*, and *Onychothrips tepperi*, exhibit very low rates of *Koptothrips* parasitism. This interspecific variation in kleptoparasitism rates may be due in part to the different life-histories of the lineages involved. In some species, such as *K. rugosus* and *K. ellobus*, the life-cycle appears to be quite short relative to the other gall-inducers (several months, compared to over nine months in other taxa), and the offspring of the foundress apparently disperse from the gall as second-instar larvae and pupate in the soil (B. Crespi and B. Kranz, personal observation). As a result, some of these taxa may keep kleptoparasitism rates low by escaping in time (i. e., minimizing the time that they are vulnerable to invasion). By contrast, species of *Onychothrips*, and *O. antennatus*, inhabit galls that appear to be more tightly sealed than those of other species. The galls are long-lived in these species, and offspring of the foundress all eclose within the gall. In addition, all of the gall-inducing thrips in the lineage containing *Onychothrips*, *O. antennatus*, *O. sterni*, and *K. augonsaxxos* are found on *Acacia* in the taxonomic section Juliflorae, whereas all other gall-inducing thrips inhabit *Acacia* in the section Plurinerves (Mound et al. 1996). Of all of the gall-inducers in this lineage, only *O. antennatus* on *A. adsurgens* suffers from much *Koptothrips* kleptoparasitism, and we have never found *Koptothrips* in galls of *O. sterni*, *K. augonsaxxos*, *O. torus*, or *Ony. pilbara* (Crespi, unpublished data).

Taken together, these observations suggest that gall-inducing thrips have three distinct strategies for reducing the impact of *Koptothrips*: 'hiding' (living in tightly-sealed galls, on host plants that may be less suitable for the kleptoparasites), 'fighting' (maintaining soldier morphs), or 'running' (escaping in time, via an accelerated life cycle). These latter two strategies are provided a striking parallel in gall aphids, some of which also exhibit soldiers and long-lived galls, while others lack soldiers and develop relatively rapidly (Moran 1993, Stern 1998). Our data also indicate that, in gall-inducing thrips, each of the three strategies is more or less successful in different species.

The fourth pattern shown in our kleptoparasitism data is that, in contrast to the results for *Koptothrips* kleptoparasites, almost all species of gall-inducing thrips are heavily attacked by species of parasites in other insect orders, primarily lepidopteran and dipteran larvae (see also Mound et al. 1996). Some of these species feed upon gall tissue, while others feed upon thrips eggs, and all cause a major reduction in thrips numbers, if not total reproductive failure. Behavioral observations indicate that soldiers are ineffective against lepidopteran larvae within their galls, because the larvae remain within silken, frass-covered tunnels with only their sclerotized head capsule exposed (Crespi, personal observation). Moreover, the caterpillars bite at soldiers that approach, usually removing their antennae in the process, in a behavior that, if it represents an adaptation, could be aptly termed 'sensory castration'. Analysis of the influences of the various non-thysanopteran enemies of gall thrips on their life-histories and behavior must await in-depth study of these enemies and their mechanisms of subversion.

What ecological factors might have ultimately led to the high kleptoparasitism rates, and the differences among gall-inducing thrips species in kleptoparasitism rates? Among Hymenoptera, factors promoting a high incidence of interspecific and intraspecific kleptoparasitism include high host synchrony and density, and concomitant highly seasonal environments (Wcislo, 1987; see also Petanidou et al. 1995). In accordance with Wcislo's hypothesis and results for Hymenoptera, the life cycles of almost all gall-forming thrips are normally highly synchronized by being tied to the synchronous production of new foliage, which occurs either annually in the spring, or, in highly-arid regions, unpredictably, after rare heavy rainfalls.

What can our analyses of kleptoparasitism in gall thrips tell us about host-parasite interactions in social insects in general? One of the main findings of this study, that gall thrips taxa with soldiers suffer rates of *Koptothrips* kleptoparasitism at least as high as those of related taxa without soldiers, compells reconsideration of the idea that the presence of a complex social adaptation coincides with a high success rate of that adaptation (see also Tallamy and Schaeffer 1997). Rather than viewing social behavior as an evolutionary pinnacle (e. g., Wilson 1971, 1990), perhaps it sometimes actually represents a relatively low and local adaptive peak, eroding and barely maintained as soldiers and workers fight their way uphill under an onslaught of enemies. And although we might consider this pattern as leading to a pessimistic view of life, we must recall that without such thieves, there might be no such beautiful an adaptation as cooperation in an insect so otherwise-ignoble as a thrips.

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ON RESEARCH AND ENTOMOLOGICAL EDUCATION III: FIREFLY BRACHYPTERY AND WING "POLYMORPHISM" AT PITKIN MARSH AND WATERY RETREATS NEAR SUMMER CAMPS (COLEOPTERA: LAMPYRIDAE; *PYROPYGA*)

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ABSTRACT

The origin, evolutionary malleability, and sometimes loss of insect wings, gossamer structures whose existence has reshaped the natural world, is one of the most interesting and enigmatic dramas of insect biology. Lampyridae have long been known for the reduced wings that occur in females of some genera, but in all previously known examples it is a *fait accompli*, with little or no intraspecific variation. Such variation occurs in and among populations of the little daytime firefly *Pyropyga nigricans*, and also, among these populations there appears to be variation in sexual involvement in the phenomenon, with brachypterous males also occurring at some localities. This firefly provides an opportunity for students, both in summer classes and as solitary individuals, to study the evolutionary biology of wings, from adaptive significance to sexual selection and population ecology and genetics, to speciation, and in a variety of habitats from strands on northern glacier lakes to southwestern montane stream sides and beyond, to west-coast marshes.

Key Words: Lampyridae, *Pyropyga*, brachyptery evolution, deme divergence, speciation

RESUMEN

El origen de las alas de los insectos, su maleabilidad evolutiva, y algunas veces su ausencia, son unos de los más interesantes y enigmáticos dramas de la biología de los insectos. Los Lampyridae se reconocen desde hace tiempo por las alas reducidas de las hembras de algunos géneros, y en todos los ejemplos conocidos anteriormente en esta familia las alas reducidas son un hecho con poca o ninguna variación. Sin embargo, polimorfismo en las alas ocurre entre poblaciones y entre individuos de una misma población de la pequeña luciérnaga diurna *Pyropyga nigricans*, y también dentro de estas poblaciones parece haber variación en la participación del fenómeno en la atracción sexual. Esta luciérnaga brinda una oportunidad a los estudiantes tanto en clases de verano como individualmente para estudiar la biología evolutiva de las alas desde su significancia adaptativa en la selección sexual, en la ecología poblacional y en la genética, hasta la especiación, y además en una variedad de hábitats desde las orillas de los lagos glaciares del norte hasta los bordes de riachuelos montanos en el suroccidente y más allá en los pantanos de la costa este.

In this symposium series I have passed along notes on the natural history of fireflies I have met in the field, as I might in written lectures (Letters) to an introductory biology class, in the spirit of the initial introduction by John Sivinski. I continue here with the story of a firefly that has no adult lantern nor nocturnal activity, but instead uses pheromone communication in broad daylight. This is another illustration that taxonomists—in this example the late John Wagoner Green—have valuable observa-

tions and speculations on their taxa that go unnoticed indefinitely, hidden away in esoteric papers, perhaps archived in personal field books after their authors have passed on, unless we make special effort to help them out into the open. An informed teacher can place such memorabilia in a larger biological context and use them as vehicles to introduce, sketch, and add human interest to a general subject area. Publication of such lessons makes these useful notes and essays available to others to initiate projects at several levels of biological sophistication, beginning with field exploration in particular, and should be encouraged as a legitimate method of primary publication. This is what I do here, though the background and related information is abbreviated.

In this example, a cryptic treasure buried in the revision of a small and "not especially interesting" genus of Lampyridae was recalled by a teacher/researcher ("your present author") who recognized the phenomenon in specimens collected by a student doing a summer project. It involves shortcomings, so to speak, of firefly wings and elytra, and why it is that such valuable adaptations as flight and protective body armament can be traded away or lost. The subtexts of the phenomenon, the "whys" of selection and adaptation, and the "wheres" of population divergence should invite the attention and investigation of student and professional biologists.

In known cases of wing reduction in fireflies members of a species are short-winged to approximately the same degree. In other words, the transformation events are passages of the past, and in our time each is seemingly complete, a done deed as they say. Cantharoid taxonomist Green discovered unique and perhaps yet unfinished examples when he revised the genus of "little daytime fireflies," *Pyropyga*, in 1961. The nominal species of interest occurs across North America and individuals of both sexes are typically long winged. Green's two populations in which wings were shorter than typical for this firefly were 2500 miles apart, embedded it would seem in an infinite number of local populations of long-winged individuals. In 1973 Terry Butler invited my attention to some unusual specimens that she had found along the shore of Douglas Lake in northern Michigan, at the University of Michigan Biological Station (UMBS), near Pellston. With this as introduction, let us begin the lesson . . . after this brief message: The Internet (electronic) publication of this paper has additional figures as InfoLink attachments to illustrate the text; these are color slides of the fireflies and their sites. These are cited in text here by their number as ILR figures. Legends for InfoLink figures are included here in this printed version in the End Notes section. These copyrighted illustrations may be used freely with this citation: J. Lloyd, Univ. of Florida.

Letter XIX

On Becoming A Glowworm—The Disappearance Of Firefly Wings and Flight, Over Time and Space (Lampyridae: *Pyropyga nigricans*)

> When I am working on a problem, I never think about beauty. I think only how to solve the problem. But when I have finished, if the solution is not beautiful, I know it is wrong.
>
> (R. Buckminster Fuller, architect)

Dear Fireflyers, The wings of insects fascinate many entomologists before their futures catch up with them and they become entomologists. I can imagine that soon after the painful light of conscious thought first glimmered in a hominids head, he and she envied the wings of dragonfly and butterfly, for with them they would not have to walk over rough ground all the way to a watering or wintering place. Entomologists

attribute some of the great success of their beloved subjects to wings, whether success is measured by the phenomenal number of species or the equally unbelievable number of life-styles and niches taken by them, or by their diversity of form. Insects do more than fly with their wings. They rub them and broadcast rap, they wave them and push molecules of sex pheromones toward potential mates, and in southeast Asia tree-swarming fireflies use them as upper jaws of clamps that hold partners tightly, against intrusions of pushy interlopers perched all 'round (Fig. 1).

With the adaptive advantages offered by wings, one must wonder why it is that over evolutionary/geological time the females of several firefly species have greatly reduced and sometimes even lost theirs. How could such a conspicuous handicap be favored by natural selection? Unfortunately, in known cases of wing reduction in fireflies all members of a species are short-winged to approximately the same degree. This means that in each of these lineages the happening is in the past, and we can only observe products, not the process as it is occurring. Probably this is to be expected, for it may require only a few tens or hundreds of generations to go to completion.

But, remarkably, there is one North American firefly that today, even now as you read this, appears to be in the process of losing its wings, and this reduction seems to be proceeding differently, to have reached a different condition in each of the few local populations presently known to exist. If this is correct, this firefly is a living model for evolution/adaptation studies, with something to teach us about how wings may sometimes be lost by fireflies. It may also show us how the gene pools of local populations may become isolated from nearby parent populations, with each being a living experiment and a unique step in a passage of possibility toward becoming a new species.

Fig. 1. Copulation clamp employed by a male of *Pteroptyx valida* in a firefly tree near Bangkok, Thailand. The tip of the male's elytra are pushed under those of his female (at right) and tightly down against the top of her abdomen; at the same time the tip of his abdomen is pushed up against hers from below, holding her in a vice-like grip.

The named species of promise is *Pyropyga nigricans* (Say) (Fig. 2), and as presently understood, this little daytime firefly occurs across northern United States and southern Canada, and southward in the west into Mexico (Fig. 3). My education by this firefly began in 1973 when Terry Butler, a student doing a project under my direction at the University of Michigan Biological Station collected some remarkable specimens with much-shortened wings along the shore of the "Bug Camp's" Douglas Lake (Fig. 4). When I saw them I recalled that master taxonomist John Wagoner Green (Fig. 5) had mentioned this phenomenon in his 1961 taxonomic revision of the genus *Pyropyga*. In the section on *P. nigricans* he noted:

> "In an interesting series [of specimens] collected by Peter Rubtzov at Pitkin Marsh in Sonoma County, California, the elytra in both sexes are definitely shortened, exposing several abdominal segments. In another series, collected by the author on the shores of Lake Champlain, near Plattsburg, NY, the same incipient brachyptery [short wingedness] is evident in the females but not in the males. Possibly this phenomenon is associated with permanent moisture." (page 68)

The Pitkin marsh fireflies were collected during a botanical survey of the marsh in 1951-52. In 1990 Rubtzov sent me photos and additional information about the site; Fig. 6 shows the spot where the fireflies were abundant. He wrote: "(the beetles were especially numerous in an open, marshy area with very wet, soggy ground covered by sedges and other wetland herbs . . . there was *no* significant open water . . . only a very narrow, sluggish creek, overgrown by wetland vegetation, in the vicinity)" Green's own Lake Champlain locality was probably a cobble beach, such as or perhaps even the same one shown in Fig. 7 (ILR 1999, Fig. 1), where I found the fireflies in June 1998, 62 years after Green collected his series of specimens.

To put a repeatable, quantitative method into the evaluation of the wing-reduction phenomenon, measurements are needed. This presents two problems, but both seem to be manageable: (1) to see and measure flight wings of preserved dry specimens, they first must be softened (relaxed), then one wing removed from beneath its elytron, unfolded, and placed on a microscope slide. Fortunately there is a strong correlation between elytra and flight wing lengths (Fig. 8). Thus, elytral length can be used as a rapid and reliable indicator of flight wing length, and no dissection or specimen mutilation is needed. (2) Flight wing and elytron lengths vary with specimen size; thus, their lengths must be calibrated for overall body size. To do this, I divided the elytral length of a each specimen by that specimen's pronotal width—body dimensions are commonly used for such calibration in taxonomic keys (see sketch in Fig. 9). I will use this ratio (quotient) to compare wing reductions among *P. nigricans* specimens of diverse body sizes. (Ear lengths in certain breeds of show dogs, when laid forward must not reach the nose, to demonstrate appropriate "conformation to breed"!)

I borrowed and measured Greens two series of specimens from Pitkin Marsh and Lake Champlain. In Fig. 9 note the vertical dotted line at ratio 2.25, which I placed to separate Greens short- and long-winged specimens, cueing on and quantifying the evaluation he made. I will use this line for reference in charts of measured *P. nigricans* from other localities. What initially made Green's discovery especially interesting, in addition to the virtual certainty that his two populations had not been in genetic contact for some geological time, was that there was an apparent sexual difference in the occurrence of brachyptery. Let your mind run with this for a moment—does this indicate significant differences between the two populations in alleles, genes, strength of selection favoring brachyptery, immigration and the degree of isolation from neighboring demes, mate choice and sexual selection, number of generations since the initial appearance of brachyptery in each population, stage of

Fig. 2. Habitus of *Pyropyga nigricans* (Say), a carbon dust drawing by Laura Line. This firefly was named *Pyropyga fenestralis* by Melsheimer in 1846, but Thomas Say's name of 1823 has priority (see Green 1961).

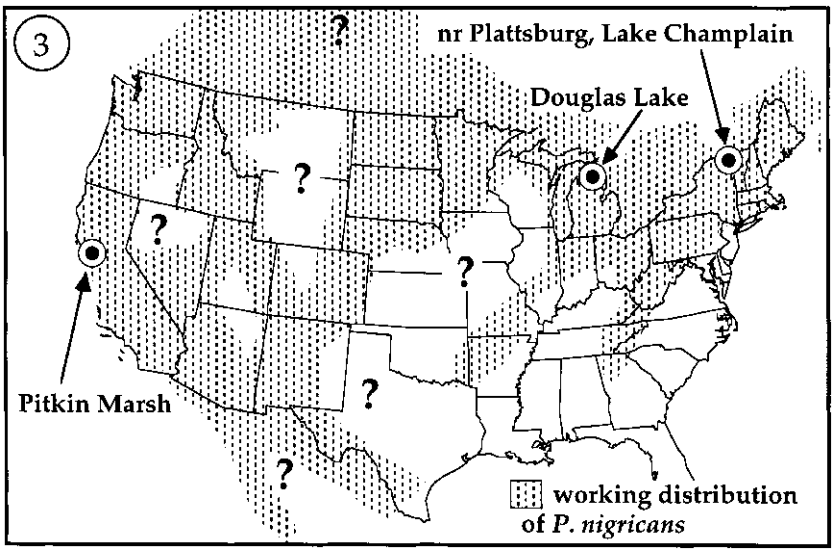


Fig. 3. Distribution map of *Pyropyga nigricans*, with general distribution based on locality labels of identified specimens. Green's two localities, Pitkin marsh in Sonoma County, CA and Plattsburg on Lake Champlain in Clinton County, NY, and the location of Douglas Lake in Cheboygan County in northern Michigan, are indicated. Question marks indicate areas of uncertainty of occurrence — perhaps only temporary gaps in my specimen data.

ecological succession of the site . . . or is it merely the result of sampling error (i.e., Green's small samples)?

Figure 10 shows the elytral ratios of specimens that Butler and I collected and measured from various locations along the shoreline around Douglas Lake in 1973, and Fig. 11 shows ratios of a sample I made 25 years later (ILR 1999, Fig. 2). Note that the sexual involvement is different from that observed in either of Green's two samples, that the female ratio is bimodal (has two peaks) with separation falling near Green's line, and that male ratios range broadly but never as low as those of females. This pattern is also shown by Cheboygan County specimens that are archived in the University of Michigan Museum of Zoology (Fig. 12). These specimens were collected between 1917 and 1969, many from the Douglas Lake vicinity.

Are there more variations around unexplored lakes and marshes in North America? In the course of identifying fireflies for several museums I have viewed many specimens of *P. nigricans* and measured some of them, to have size records, and have found a few other brachypters. Some were archived in the American Museum (NYC) collection, and were collected in 1961 and 1964 at McMillan Camp near Silver City, NM, by lepidopterist Frederick Rindge and his family (Fig. 13). Specimen labels indicated that they were collected at 6800 feet elevation. Rindge replied to my letter of habitat inquiry, after consulting his field notes, that the camp was "situated in a rather small river bottom, with a profusion of ponderosa pine, oak and junipers, plus a great assortment of smaller trees and shrubs. But being in this rather narrow canyon, the stream was always nearby." This location sounds to me as though it shares features with shoreline strands, with unfriendly and isolating habitats on each side! Figure 14 shows the ratios of all of the other North American specimens I have measured.

Fig. 4. A brachypterous female *P. nigricans*, originally photographed for me by Gary Williams at the Bug Camp in 1973; this print was made from the original and is of lesser quality. Note that the dorsal tip of her abdomen (pygidium) is narrowly rounded; those of males are truncate. Her elytral ratio is 1.6.

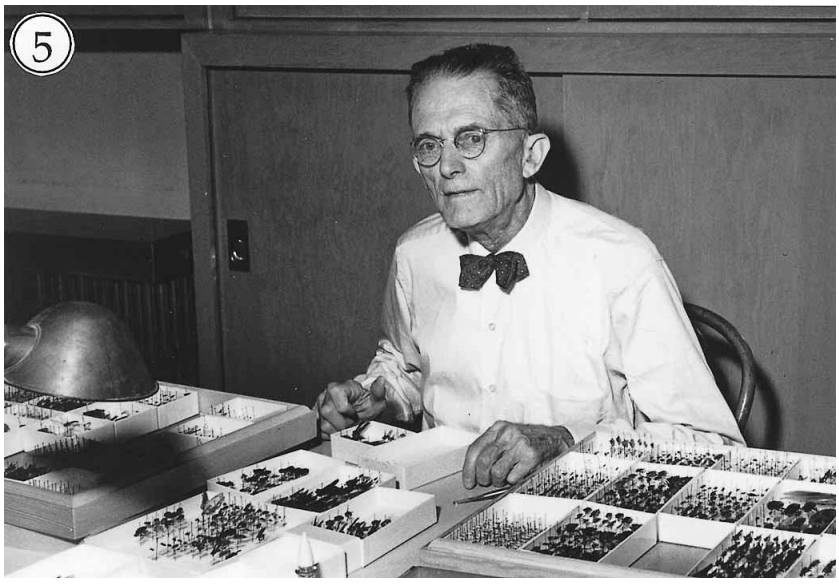


Fig. 5. Taxonomist John Wagoner Green at his desk, about 1960. This photo was provided by the California Academy of Sciences, where Green had taxonomized.

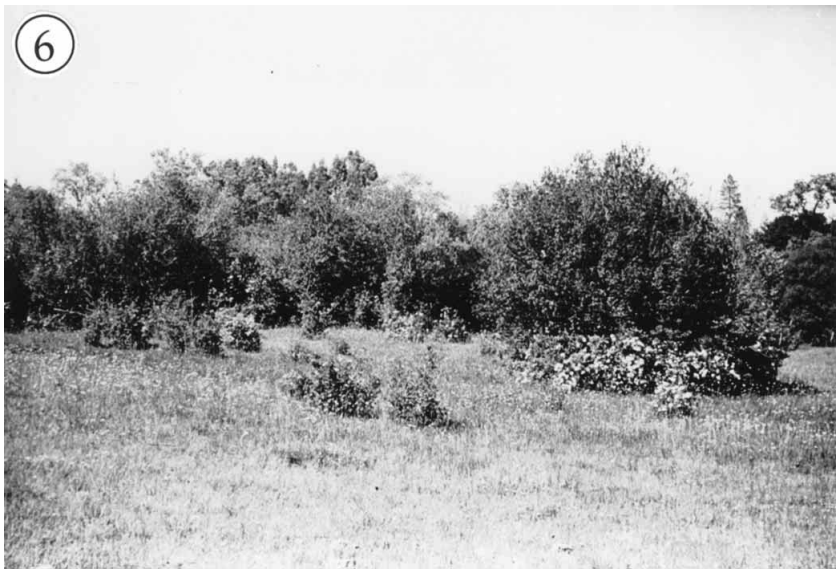


Fig. 6. *P. nigricans* site in Pitkin Marsh, Sonoma County, CA; this photograph was taken by the late Prof. William Hovanitz and provided to me by botanist Peter Rubtzov (see text). Fireflies were most numerous in the area in front of the large shrub at the right.

Fig. 7. *P. nigricans* 1998 site near Plattsburg, NY on Lake Champlain. Green's site was near, perhaps even this one. Fireflies occurred within a few feet of the water, on sand and cobbles. This print was made from a color slide, and lacks the quality that a monochrome negative would have given.

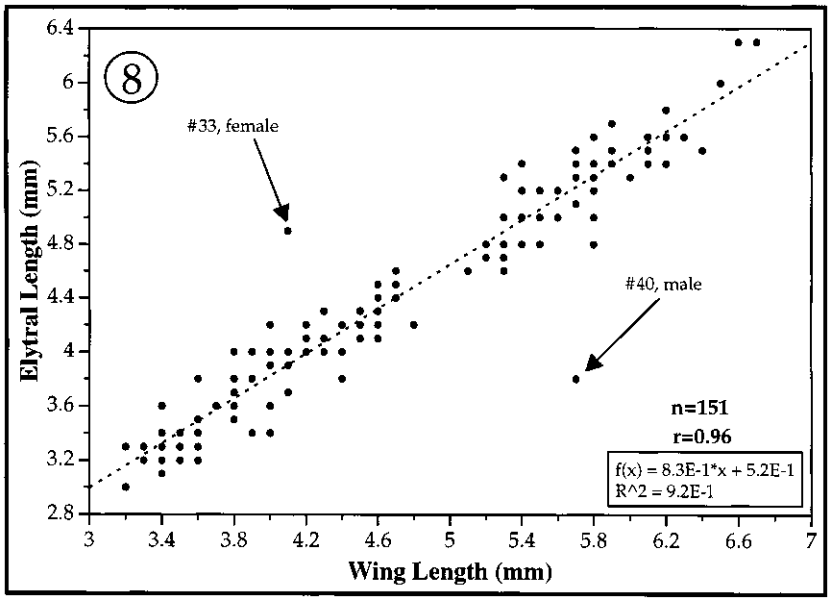


Fig. 8. Elytron length as a function of wing length, showing their strong correlation. This permits the easily measured elytral length to be used to assess wing reduction. Measurements were made by Terry Butler and me.

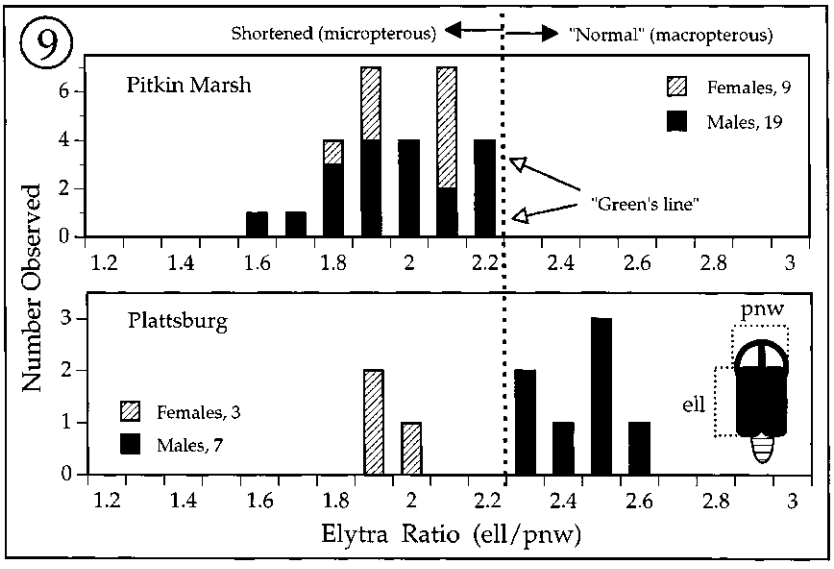
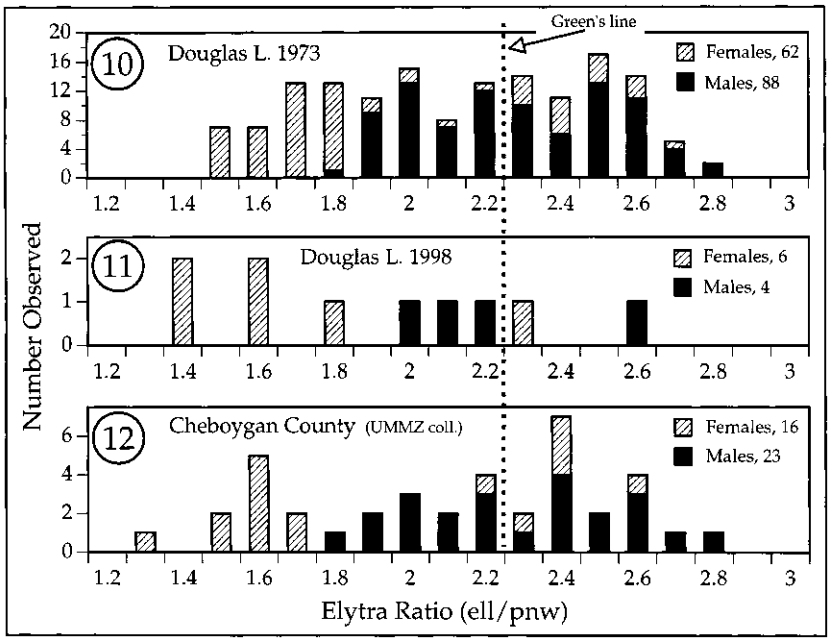


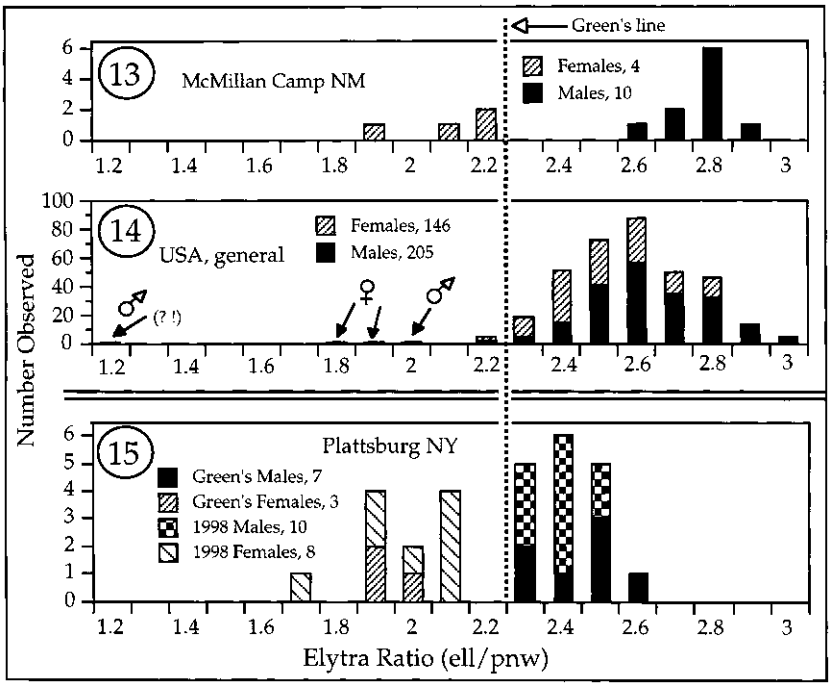
Fig. 9. Quantification of elytral reduction occurring in each of Green's specimen series. Elytra ratio is the quotient of elytral length divided by pronotal width; note sketch. Note that sexual involvement is different in the two samples.



Figs. 10-12. Quantification of elytral reduction in Douglas Lake fireflies and vicinity. Elytra ratio = elytral length/pronotal width. Note that sexual involvement is different from that seen in Green's two samples, shown in Fig. 9 (see text).

Now I excitedly ask, with anticipation, if we are seeing wing length in evolutionary transition, are there brachypterous populations of different ages out there to be sampled for comparison, to be found by wading around marshes and lakes in old tennis shoes? One especially interesting exploration would be to follow the outlet of Douglas Lake—the Maple River—and see whether (a younger population of?) brachypters occur at its mouth where it enters Burt Lake on its water's way to the St. Lawrence and Atlantic (ILR 1999, Fig. 3). I found none where I looked near a boat ramp, nor at another and unspoiled but accessible strand on this lake. Recalling Green's personal field discovery, Lake Champlain has a long shoreline and many islands and streams. My 1998 sample from near Plattsburg is similar in ratio to his 1936 sample (Fig. 15). The map in Fig. 16 shows suspicious localities identified by ratio values (ratios < 2.25) shown in Fig. 14.

There are many places to look, when you consider all of the thousands of glacier lakes advertised by Minnesota, Wisconsin, and Michigan, to say nothing of lakes and canyons scattered throughout the general range of *P. nigricans* (ILR 1999, Fig. 4). Over the past century geologists have learned that the space that became Douglas Lake began as a large, long-lasting chunk of ice, broken from the terminal end of a melting, brittle glacier, leaving a pit (kettle) in the gravel, and that what is now firefly shoreline has been developing in wind and waves and a changing water level for 9500 years. They also know that the climate has changed from cold and damp to warm, and the surrounding forests, from spruce to pine to oak and other hardwoods. They also tell us that Douglas Lake will eventually drain out the Maple River to Burt Lake. So many lakes, so much happening, so little time . . .



Figs. 13-15. Charts showing (13) elytral reduction in *P. nigricans* from a site in New Mexico; (14) elytra ratio in a general sample of *P. nigricans*; and (15) a 1998 Plattsburg sample combined with Green's original sample. Elytra ratio = elytral length/pronotal width. (see text)

I made a few observations on *P. nigricans*' mating behavior at Plattsburg and Douglas Lake. Mating occurred from sunrise to midday, with males and possibly females too being attracted to female pheromones (Fig. 17; ILR 1999, Fig. 5). Adults remained within a few feet of the shoreline and after coupling they turned tail-to-tail; though tiny, pairs were conspicuous on sand, gravel, and stones (ILR 1999, Figs. 6 and 7). Winged males rarely flew, and when they did their flights were short, typically less than a meter in length; I saw only one flying in 1998. Figures 18-20 show activity "profiles" made along beaches at the two localities. Larvae were found walking along beaches at Douglas Lake within a meter of the waterline on damp sand (ILR 1999, Fig. 8). I never found nocturnal activity by juveniles or adults.

To conclude and highlight, questions of natural selection happily arise—why are individuals with shorter wings better at reproducing, at leaving offspring with their alleles in such ecological situations, than are individuals with longer and flight-capable wings? This phenomenon in insects has been noted and considered by a succession of naturalists for more than a century. The strand habitat, that is, the shorelines of lakes, rivers, and oceans, and around islands, has often been associated with wing reduction and loss. Among possibilities that have been considered and that could fit here: if this firefly gains little or nothing from flight, allelic substitutions from strong selection in pleiotropic contexts could substitute alleles that produce reduced wings; energetic savings realized by not building wings could be diverted into eggs or mate

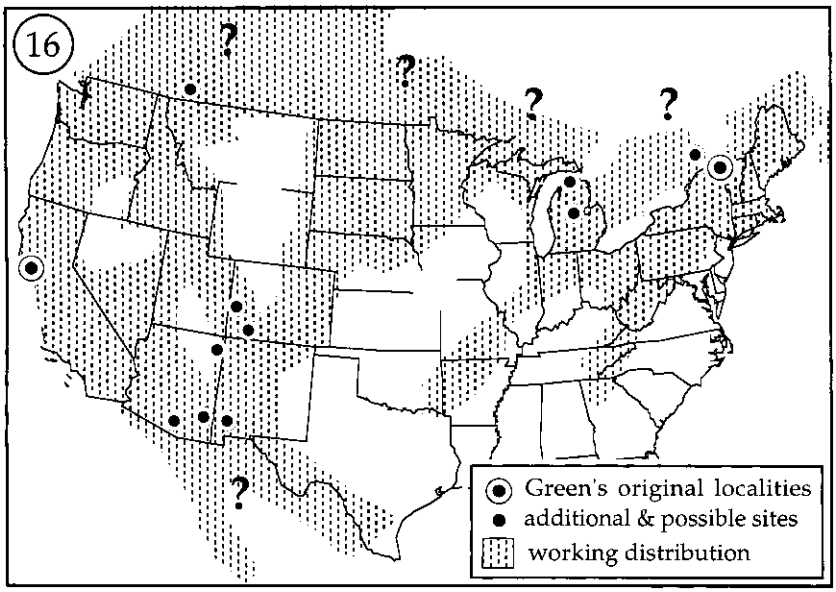


Fig. 16. Known and suspected *P. nigricans* brachypter locations. Isolated lakes and montane canyons are promising situations.

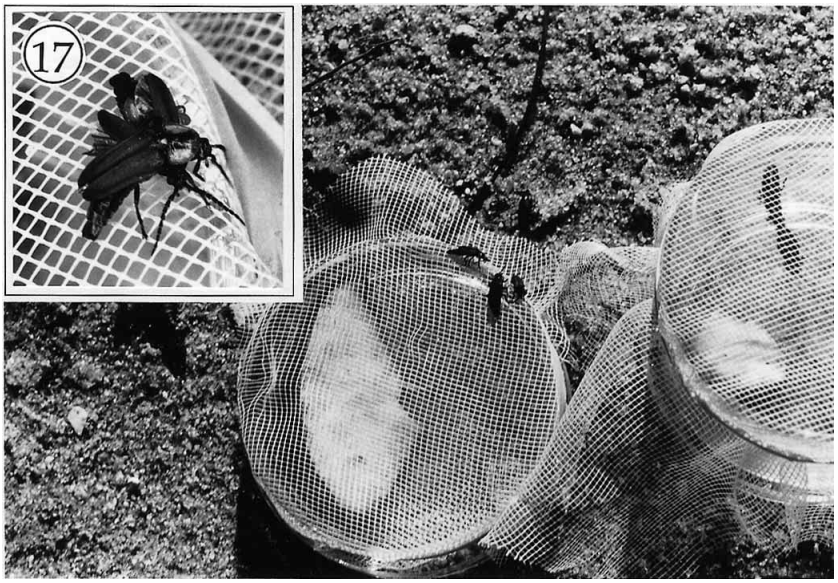
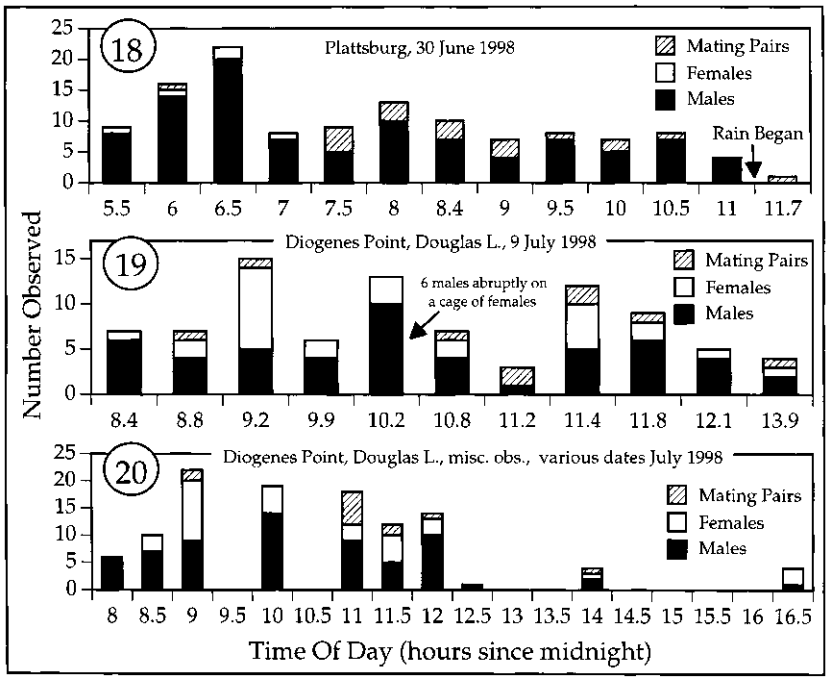


Fig. 17. When females were placed in a net-covered dish on the Douglas Lake beach males and females quickly approached (appeared) and walked up onto the net. Inset shows male atop another, and their spatulate pygidia. Red mites are common on these fireflies; there is one in the inset, spreading the lower male's wings.



Figs. 18-20. Adult activity profiles for two sites. (18) A systematic census in the site at Plattsburg; (19) a systematic census at a site on Douglas Lake; (20) a nonsystematic collection of incidental counts made on several days at the Douglas Lake site.

search (and provide an advantage over short geological time); because flyers can be blown over open water away from limited or narrow habitats, having wings may often be fatal (a genetic lethal!) in such situations.

Of special interest in strand inhabiting *P. nigricans*, is whether their genetic isolation from nearby, say, just-inland demes is primarily geographical (spatial), or if mate choice and sexual selection have become involved and promote genetic isolation. This consideration properly enlarged brings fireflyers into the realm of sympatric speciation models, which, in my view, is a too-neglected aspect of taxonomic thought for insect fancying naturalists afield.

Perhaps it will be found that the population of fireflies in Pitkin Marsh, interpreted for sake of mental jogging as nearing maturity, has proceeded further toward wing reduction stability than other *P. nigricans* now known. Maybe this population is very old and began somewhere else, within walking distance of course, on a strand around a now dried up pond or lake? Surely, when we have more data on these little daytime fireflies, and now I explain this letter's obscure title, we will understand more of the evolution of wing reduction and loss in luminous glowworm and lightningbug fireflies.

ENDNOTES

I thank John Sivinski, Steve Wing, and Jade Williams for reading the manuscript, and Flora MacColl for technical assistance in the preparation of the manuscript and

for preparing the InfoLink attachments; Juan Manuel Alvarez for translating the abstract into Spanish for the resumen, Peter Rubtzov for photographs and ecological information on the Pitkin Marsh site; and Frederick Rindge for ecological information on the McMillan Camp site. I thank the staff and administration of the University of Michigan Biological Station (UMBS) in 1973 and 1998, for use of their outstanding facilities and equipment, for bringing key references in their library to my attention, and for their unstinting cooperation. I especially want to thank Terry Butler, the student who saw something "peculiar" about the Douglas Lake brachypters and brought them to me, and then collected and measured many of them; Gary Williams, for taking the photograph of the firefly in Fig. 4 and teaching me the fundamentals of darkroom photography; and Jack Burch for his kind hospitality, answers to snail questions, and barge piloting during the 1998 survey of sites along the circumference of Douglas Lake.

Archived specimens from several collections have been viewed over several years, and I thank each of the curators and collection managers for loan of these specimens. Their institutional affiliations and collections are here indicated by name and "Arnett coden": Lee H. Herman, American Museum of Natural History (AMNH); Mark F. O'Brien and Richard D. Alexander, Museum of Zoology, Univ. of Michigan (UMMZ); Hugh Powell, Staten Island Museum; Norman D. Penny, California Academy of Sciences (CASC); Jerry Pilney and Alan Morgan, Dept. of Earth Sciences, Univ. of Waterloo, Canada; Robert E. Lewis, Dept. of Entomology, Iowa State Univ. (ISUI); Brett C. Ratcliffe and Charlie Messenger, Systematics and Research Coll., Univ. of Nebraska (DEUN); the late Floyd G. Werner, Dept. of Entomology Coll., Univ. Arizona (UAIC); Roland L. Fischer, Michigan State Univ. Coll. (MSUC); Bruce Gill, National Museum of Natural Sciences (CNCI), Canada; Robert H. Turnbow, Jr., Dept. of Entomology Coll., Univ. of Georgia (UGCA).

The following enumerated statements are figure legends for color illustrations (slides) that appear as InfoLink attachments to this article in the electronic publication of this issue of the *Florida Entomologist*, and which are cited in text here as ILR 1999, Fig.#: 1. The strand on Lake Champlain near Plattsburg where I made behavior observations in June 1998, and probably near and similar to Greens 1936 collection site. 2. Diogenes Point on Douglas Lake; the July 1998 observation site was the open strand seen to the right. 3. The Maple River, looking downstream just inside the outlet at the southwest corner of Douglas Lake, where the stream begins its woodsy flow to Burt Lake, 118 feet lower in elevation and a mile and a half in distance. 4. A stony strand on the Ontario side of the Ottawa River in Canada, at about 46 N Latitude; to my eye it looks much like the Plattsburg locality, but I found no *P. nigricans*. 5. A shorter-winged male *P. nigricans* that has been attracted to a cage of females. Note the spatulate pygidium that readily identifies him as a male, and a female's silhouette in the cage below the net. He feeds at least four red mites (Acarini), common parasites of shoreline insects. 6, 7. Coupled pairs of *P. nigricans* on a cobble and on a twig on the shore of Lake Champlain. Such pairs are easily spotted, and some (all?) remain coupled for hours. 8. A wind-swept beach on North Fish Tail Bay, Douglas Lake, where 14 larvae were seen (hunting?) along three feet of the shoreline on damp sand; all were within two feet of the waters edge. Florida Agricultural Experiment Station Journal Series Number R-06817.

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KLEPTOPARASITISM AND PHORESY IN THE DIPTERA

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ABSTRACT

Spiders, dung-feeding scarabs, social, and prey-storing insects provide predictable and concentrated sources of food for a variety of thief flies (kleptoparasites) and their larvae. Whenever waiting in the vicinity of the "host" for an opportunity to exploit its resources is more energy efficient and less dangerous than foraging among hosts, a number of intimate relationships between the fly and host may evolve. In extreme cases, flies may become long-term phoretic associates that travel with hosts even while the latter is in flight. The behaviors and ecologies of kleptoparasitic Diptera are

reviewed with special attention paid to the adaptations of Sphaeroceridae phoretic upon Scarabaeidae. The mating systems of kleptoparasitic flies are influenced by the type of resource that is stolen; flies associated with predators are mostly female, while those found on scarabs are of both sexes. These differences are discussed in terms of mate location, sperm competition, and mate choice.

Key words: Sphaeroceridae, Milichiidae, Chloropidae, mating system, mate choice

RESUMEN

Para una variedad de moscas ladronas (cleptoparasíticas) y sus larvas, las arañas, escarabajos peloteros, insectos sociales e insectos que almacenan sus presas son fuente de alimento predecible y concentrada. Siempre que sea más eficaz y menos peligroso el esperar en la cercanía del hospedero para aprovecharse de sus recursos en lugar de buscar alimento entre los hospederos, varias relaciones ecológicas íntimas entre la mosca y su hospedero podrían evolucionar. En casos extremos, las moscas pueden volverse socios foréticos, viajando con sus hospederos mientras éstos vuelan. Se examinan el comportamiento y la ecología de los dípteros cleptoparasíticos dándole atención especial a las adaptaciones de las moscas foréticas Sphaeroceridae en Scarabaeidae. El sistema de apareo de las moscas cleptoparasíticas es influenciado por el tipo de recurso que se robe; las moscas asociadas con depredadores son en su mayoría hembras, mientras que aquellas asociadas con escarabajos son de ambos sexos. Se discuten estas diferencias en cuanto a la localización de la pareja, competencia de la esperma, y la elección de la pareja.

Thus what the world calls good business is only a way
To gather up the loot, pack it, make it more secure
In one convenient load for the more enterprising thieves.

Chuang Tzu, 250 B.C.

In this unpredictable and competitive world, many arthropods have found it adaptive to sequester, and sometimes personally guard, resources for their future use or the use of their offspring. For example, some dung beetles spend many hours shaping, moving, burying, and shielding a fecal fragment they may either eat, or into which they may lay an egg (Halffter & Edmonds 1982). Caching results in a delay between taking possession of a resource and its final consumption, and during this period owners are vulnerable to thieves (kleptoparasites). Other invertebrates at risk from kleptoparasites simply take a relatively long time to consume their food; e.g., certain web-building spiders may take minutes to hours to masticate and preorally digest a victim. Again, delay exposes predators to thieves, and the robbers that exploit both cachers and slow-eaters are often small Diptera.

A number of these tiny kleptoparasitic flies have intimate relationships with their larger "hosts" (Table 1). Some are phoretic and spend hours or days upon the bigger animal, not feeding, but simply waiting for it to obtain the item the fly wishes to share. In such cases it is presumably more efficient and less hazardous to wait for a particular host to obtain something valuable than it is to search for a host that happens to be feeding or burying at that particular time. Other flies wait near their host and can join it in an instant. The reasons for closeness are similar to those that have lead to phoresy, but perhaps the lack of mobility inherent in phoresy or the danger of continually clinging to a giant has resulted in a looser association. In still other instances kleptoparasites search out "wealthy" arthropods and contacts are brief and sporadic.

TABLE 1. REPRESENTATIVES OF VARIOUS FORMS OF KLEPTOPARASITIC FLIES AND WHETHER OR NOT THEY PRACTICE PHORESY. P = PHORESY, CA = CLOSE ASSOCIATION, I.E., FLIES REMAIN IN THE VICINITY OF THE "HOST" BUT NOT UPON IT, A "?" AFTER A NOTATION REFLECTS A BELIEF THAT EITHER PHORESY OR CLOSE ASSOCIATION IS PRACTICED BUT THAT UNEQUIVOCAL OBSERVATIONS ARE MISSING, AND A "." REPRESENTS AN ABSENCE OF DATA.

Species	Host	Type of Association	Citation
Adult kleptoparasites of social insects (food thieves)			
Braula coeca	*Apis mellifera*	P	Askew 1971
Vestigipoda myrmolarvoidea (Phoridae)	*Aenictus* sp. (Formicidae)	CA	Disney 1996
Termitophodrides heterospinalis (Phoridae)	*Cornitermes similis* (Isoptera)	P	Bristowe 1924
Termitoxeniinae (Phoridae)	Isoptera	CA	Disney & Kistner 1997
Malaya spp. (Culicidae)	Formicidae	CA	Farquharson 1918
Adult kleptoparasites of predatory arthropods (food thieves)			
Gaurax sp. (Chloropidae)	Araneidae	P	Bristowe,1941, Ismay 1977
Phyllomyza sp. (Milichiidae)	*Nephila clavipes* (Araneidae)	P	Robinson & Robinson 1977
Phyllomyza sp. (Milichiidae)	*Nephila clavipes* (Araneidae)	P (?)	Sivinski & Stowe 1980
	Rhinocornis cuspidatus (Reduviidae)		
	Misumena vatia (Thomisidae)		
Desmometopa sorida (Milichiidae)	*Argiope bruennichii* (Araneidae)	P	Richards 1953
	Argiope argentata (Araneidae)	P	Robinson & Robinson 1977
Conioscinella spp. (Chloropidae)	*Scolopendra veridis* (Scolopendridae)	P	Sivinski 1985
Neophyllomyza wulpi (Milichiidae)	*Ommatius minor* (Asilidae)	P	Biro 1899
Anomoeoceros punctulatus (Chloropidae)	Araneidae	P/CA	Ismay 1977
Olcella cinerea (Chloropidae)	*Nephila clavipes* (Araneidae)		
	Reduviidae	CA	Sivinski 1985
Olcella quadrivittata (Chloropidae)	Asilidae		
	Mantodea	CA	Marshall 1998
Olcella trigramma (Chloropidae)	Reduviidae	.	Sivinski 1985

TABLE 1. (CONTINUED) REPRESENTATIVES OF VARIOUS FORMS OF KLEPTOPARASITIC FLIES AND WHETHER OR NOT THEY PRACTICE PHORESY. P = PHORESY, CA = CLOSE ASSOCIATION, I.E., FLIES REMAIN IN THE VICINITY OF THE "HOST" BUT NOT UPON IT, A "?" AFTER A NOTATION REFLECTS A BELIEF THAT EITHER PHORESY OR CLOSE ASSOCIATION IS PRACTICED BUT THAT UNEQUIVOCAL OBSERVATIONS ARE MISSING, AND A "." REPRESENTS AN ABSENCE OF DATA.

Species	Host	Type of Association	Citation
Trachysiophonella pori (Chloropidae)	*Zodarium frenatum* (Zodariidae)	CA	Harkness & Ismay 1975
Paramyia nitens (Milichiidae)	*Argiope aurantia*	CA	Sivinski & Stowe 1980
	Nephila clavipes (Araneidae)	(?)	Eisner *et al*. 1991
Neophyllomyza spp. (Milichiidae)	*Nephila clavipes* (Araneidae)	.	Robinson & Robinson 1977
	Zelus trimaculatus (Reduviidae)	.	Sivinski & Stowe 1980, Eisner *et al*. 1991
Milichiella sp. (Milichiidae)	*Nephila clavipes* (Araneidae)	CA (?)	Sivinski 1985 Eisner *et al*. 1991
Desmometopa m-atrum (Milichiidae)	Araneida	.	Mik 1898
	Reduviidae	.	Biro 1899
Desmometopa singaporensis (Milichiidae)	Araneida	.	Mik 1898
	Reduviidae	.	Biro 1899
Desmometopa m-nigrum (Milichiidae)	*Thomisus onuustus* (Thomisidae)	.	Knab 1915
Desmometopa latipes (Milichiidae)	*Phidippus multiformis* (Salticidae)	.	Frost 1913
Desmometopa sp. (Milichiidae)	*Nephila clavipes* (Araneidae)	CA (?)	Eisner *et al*. 1991
Didactylomyia longimana (Cecidomyiidae)	*Nephila clavipes* and other Araneida	CA	Sivinski & Stowe 1980
Culicoides bauri (Ceratopogonidae)	*Nephila clavipes* (Araneidae)	.	Sivinski & Stowe 1980
Atrichopogon sp. (Ceratopogonidae)	Araneidae	.	Downes & Smith 1969
Microphor obscurus (Empididae)	Araneidae	.	Downes & Smith 1969
Microphor crassipes (Empididae)	Araneidae	.	Laurence 1948
Megaselia sp. (Phoridae)	*Nephila clavipes* (Araneidae)	.	Sivinski & Stowe 1980
Lonchaea chorea (F.) (Lonchaeidae)	*Enoplognatha ovata* (Clerk) (Theridiidae)	.	Dobson 1992

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Species	Host	Type of Association	Citation
Lonchaea laticornis Meig. (Lonchaeidae)	*Enoplognatha ovata* (Theridiidae)	.	Dobson 1992
Setisquamalonchaea fumosa (Egger) (Lonchaeidae)	Araneida	.	Dobson 1992
Larval kleptoparasites of oviposition ingress (thieves of developmental resources)			
Taeniostola limbata Hendel (Tephritidae)	*Cyrtotrachelus* sp. (Curculionidae)	P	Kovac & Azarae 1994
Larval Kleptoparasites of social insects (thieves of developmental resources)			
Myrmecophilous Phoridae	Formicidae	CA	Disney 1994
Cataclinusa pachycondylae (Phoridae)	Formicidae	P	Wheeler 1910
Larval kleptoparasites of prey storing insects (thieves of developmental resources)			
Miltogrammine (Sarcophagidae)	Sphecidae, Vespidae	CA	Evans 1966
Lepidophora spp. (Bombyliidae)	Sphecidae, Vespidae	.	Hull 1973
Lasiopleura grisea (Chloropidae)	*Bembix cameroni* (Sphecidae)	.	Evans 1973
Larval kleptoparasites of dung-feeding scarabs (thieves of developmental resources)			
Ceroptera rufitarsis (Sphaeroceridae)	*Scarabaeus sacer* (Scarabaeidae)	P	Lesne 1896
Ceroptera sivinskii (Sphaeroceridae)	*Geotrupes egeriei* and others (Scarabaeidae)	P	Sivinski 1983
Ceroptera longicauda (Sphaeroceridae)	*Peltotrupes pofundus* *Mycotrupes gaigei* (Scarabeidae)	P	Marshall & Montagnes 1988 J. S., pers. obs.
Ceroptera longiseta (Villeneuve) (Sphaeroceridae)	*Pachylomera* sp. (Scarabaeidae)	P	Roubaud 1916
Ceroptera nasuta (Villeneuve) (Sphaeroceridae)	*Catharius* sp. (Scarabaeidae)	P	Roubaud 1916
Ceroptera equitans (Collin) (Sphaeroceridae)	*Scarabaeus gangeticus* (?) (Scarabaeidae)	P	Fletcher 1909, Collin 1910
Biroina myrmecophila (Sphaeroceridae)	*Cephalodesmius armiger* (Scarabaeidae)	P	Montieth & Storey 1981
Norrbomia lacteipennisi (Sphaeroceridae)	Scarabaeidae	P	Steyskal 1971

TABLE 1. (CONTINUED) REPRESENTATIVES OF VARIOUS FORMS OF KLEPTOPARASITIC FLIES AND WHETHER OR NOT THEY PRACTICE PHORESY. P = PHORESY, CA = CLOSE ASSOCIATION, I.E., FLIES REMAIN IN THE VICINITY OF THE "HOST" BUT NOT UPON IT, A "?" AFTER A NOTATION REFLECTS A BELIEF THAT EITHER PHORESY OR CLOSE ASSOCIATION IS PRACTICED BUT THAT UNEQUIVOCAL OBSERVATIONS ARE MISSING, AND A "." REPRESENTS AN ABSENCE OF DATA.

Species	Host	Type of Association	Citation
Norrbomia frigipennis (Sphaeroceridae)	Many species of Scarabaeidae including the genera *Canthon, Phanaeus,* and *Onthophagus*	P	Sivinski 1983
Norrbomia singularis (Sphaeroceridae)	*Canthon* spp. and *Copris* spp. (Scarabaeidae)	P	Sivinski 1983
Pterogramma sp. (Sphaeroceridae)	*Canthon pilularius, Phanaeus* spp. and others (Scarabaeidae)	CA	Sivinski 1983

In this examination of thievery and phoresy we first review the various forms of kleptoparasitism, and make a distinction between flies that feed upon the resources of the host (adult kleptoparasites) and those who put their offspring in a position to steal (larval kleptoparasites). Particular attention is called to those species that practice phoresy and other forms of close association, and the advantages and difficulties of staying near the host are discussed. We point out that phoretic kleptoparasites sometimes accumulate in high densities on hosts, and that this intimacy has affected other parts of the flies' natural history, notably their sexual behaviors. The various mating systems of phoretic kleptoparasites are compared and contrasted, and hypotheses are offered about the roles of mate searching, mate choice, and sperm competition in their evolution. Finally, we consider the vulnerability of different types of resources, and whether accessability has influenced the diversity of various kleptoparasite guilds.

Adult Kleptoparasites (Particularly of Predaceous Arthropods)

Certain invertebrate predators are untidy eaters whose insect prey may be dripping with hemolymph and digestive secretions, and torn open to expose organs and fats. Such soups are repasts for various milichiid and chloropid flies, who lick up fluids either from the surface of the prey or from the predator's jaws (Fig. 1).

Spiders are the most commonly noted mounts of phoretic kleptoparasites. The chloropid *Guarax* sp. has been collected from orb-web spiders (Bristowe 1941, Ismay 1977), and a group of eleven Panamanian milichiids, *Phyllomyza* sp., was observed on the cephalothorax of the araneid *Nephila clavipes* (L.) over a period of four days (Robinson & Robinson 1977). Another (?) *Phyllomyza* sp., was found upon *N. clavipes* in Florida (Sivinski & Stowe 1980).

Other phoretic associations include the milichiid *Desmometopa sorida* (Fallén) which rides the backs of reduviids (Richards 1953) and a Florida chloropid, *Conioscinella* sp. mounted on the scolopendromorph centipede, *Scolopendra veridis* Say (Sivinski 1985). Biro (1899) observed up to three individuals of the New Guinean milichiid

Fig. 1. Acalypterates feeding on the hemipteran prey of the large spider *Nephila clavipes*. One individual is perched upon the chelicera of its host, while another can be seen with a fluid droplet in its mouthparts. Kleptoparasitic flies often imbibe considerable amounts and swell up to a substantial girth. (Photograph by J. S.)

Neophyllomyza wulpi Hendel (as *Desmometopa minutissima* Wulp) perched on the thoraxes of the asilid *Ommatius minor* Doleschall. By removing and marking phoretics, he discovered that they quickly remounted hosts from distances of up to 12 paces. Pairs of riding milichiids were common and they would take a position between the robber fly's wings, one facing forward and the other back (see also Kertesz 1897, Mik 1898).

The majority of adult food-kleptoparasites are not phoretic, but many seem to be closely associated with hosts nonetheless (Table 1, see Sivinski 1985 and cit.). Some species appear to wait near predators and gather at a kill in a matter of seconds. For example, the Floridian chloropid *Olcella cinerea* (Loew) can instantaneously arrive on the freshly captured prey of *Nephila clavipes* (Sivinski 1985), and *Olcella quadrivittata* (Sabrosky) can quickly find certain kinds of prey items being consumed by mantids and asilids (Marshall 1998). The cecidomyid *Didactylomyia longimana* (Felt) consumes the liquified prey of spiders and is one of the rare instances of adult feeding in the family (Sivinski & Stowe 1980). It also rests in spider webs, hanging by its front legs with its tarsi placed between adhesive droplets. While nonkleptoparasitic cecidomyids also hang in webs, the habit might have additional advantages for a kleptoparasite.

Still other kleptoparasites appear to forage widely and may not be "waiters" at all. At least some of these flies use volatiles from the defensive compounds of the prey to locate a meal. Coreidae and Pentatomidae trapped by spiders are particularly attractive to milichiids such as *Paramyia nitens* (Loew), *Neophyllomyza* sp., *Milichiella* sp., and *Desmometopa* sp. (Eisner et al. 1991, Aldrich & Barros 1995). One component of the defensive sprays of these bugs, trans-2-hexanol, attracts kleptoparasites when applied to dead moths whose bodies are not normally fed upon by the flies (Eisner et al. 1991). Other kinds of prey items, including Acanthosomatidae and Staphylinidae,

with strong defensive chemicals have also been associated with kleptoparasitic flies (Marshall 1998), but most species of kleptoparasitic acalypterates have been collected from aculeate Hymenoptera carcasses in the process of being eaten. There is also some evidence that predator digestive secretions are attractive to *Didactylomyia longimana,* and a phorid and a ceratopogonid species (Sivinski & Stowe 1980). Much remains to be discovered about chemical cues and the foraging of thief flies.

In addition to specialized kleptoparasites there are instances of certain Empididae, Anthomyiidae, and Sarcophagidae feeding in spider webs, but this feeding may be opportunistic (Irwin 1978). Recently, several species of lonchaeids have been seen partaking of spider's prey in English gardens and doing so in a careful and methodical manner that suggests specialization (Dobson 1992).

Caution is an admirable quality in a kleptoparasite that has to deal with the formidable dangers of a gargantuan predator and perhaps an entangling web. The empidid *Microphor crassipes* Macquart is a frequent prey of its spider host (Laurence 1948, see also the toll spiders take of kleptoparasitic panorpids, Thornhill 1978). *Paramyia nitens*, in spite of extremely elongate mouthparts that should aid it to safely sup between a spider's jaws, is sometimes captured and killed by its host (Sivinski & Stowe 1980). McCook (1889) found a similar fly "trussed up near the spot where it had lately fed."

The threat posed by a predator may dictate the degree of intimacy between the kleptoparasite and its host. While phoresy might allow the quickest response to a capture and be competitively superior to waiting farther away from a limited resource, it could also be more perilous. A sort of compromise may occur in the Ugandan chloropid *Anomoeoceros punctulatus* Becker which hovers, even in strong winds, directly below the chelicerae of web-building spiders (Ismay 1977).

Just as solitary predators obtain and hold desirable resources, so too do social insects which transport high-value foodstuffs from the field to their colonies. Like solitary predators, social insects also attract the attentions of kleptoparasitic flies. Phoretic adults of *Braula coeca* Nitzsch (the bee louse) feed on liquids taken from the mouths of honey bees (Askew 1971). The Brazilian phorid *Termitophodrides heterospinalis* Borgmeier is also phoretic and rides on the backs of worker termites (Bristowe 1924). Calliphoridae in the Old World genus *Bengalia* feed either as kleptoparasites or as facultative predators of various ant genera from which they snatch prey or brood (Maschwitz & Schonegge 1980). Mosquitoes in the genus *Malaya* also steal food from ants, hovering over their mouths and some cases even tapping the ant's antennae to solicit regurgitation (Farquharson 1918). Two phorid species are known to solicit food from ants (Disney 1994), and one milichiid feeds on the anal droplets of workers (Jacobson 1909). Perhaps the most remarkable association between an adult kleptoparasite and a social insect host is that of the recently discovered Malaysian phorid *Vestigipoda myrmolarvoidea* Disney and ants of the genus *Aenictus* (Disney 1996). Females of the fly are legless, wingless, larviform myrmecophiles which live in the ant colony, where they are apparently fed and cared for by the worker ants. Many Phoridae are larval kleptoparasites of ants and termites (e.g. Wilson 1971, Disney & Kistner 1997), and in many species females have greatly reduced wings, but no other phorid adults are known to be as integrated into the host social structure as is *V. myrmolarvoidea.*

Larval Kleptoparasites (Particularly of Aculeate Hymenoptera and Dung-feeding Scarabs)

Obtaining food for offspring, rather than for oneself, is the second great "motive" for kleptoparasitism by Diptera, and often for adult phoresy as well. This category is a complex one, and the nearly invariable relationship between phoresy and kleptopar-

asitism when only adults consume the resources of the host (phoretic adults are kleptoparasites) is not necessarily the case in instances where eggs are laid by phoretic adults in the vicinities of their associates (not all phoretic adults have kleptoparasitic larvae). The distinction requires some initial scrutiny, and we discuss flies that are phoretic but which probably take nothing of importance from their mounts nor compete with its offspring. Following this we examine the two best described forms of larval kleptoparasitism. First we review kleptoparasitism of aculeate Hymenoptera, and the sometimes close associations kept by the adults and larvae of such flies with their hosts. We then turn to some of the most common and easily observed kleptoparasites, the diverse and tenaciously phoretic Sphaeroceridae that ride upon dung beetles and lay eggs in the feces sequestered by their hosts.

The Reasons for Phoresy in Flies That Oviposit in the Vicinities of Other Arthropods (Kleptoparasitism vs Inquillinism). There are various "motives" for the phoretic relationships of adult flies who lay their eggs in the vicinities of other arthropods, and not all of these phoretic flies are kleptoparasites. However, in most cases of phoresy, whether involving a kleptoparasite or not, a larval resource associated with the mount is unpredictably available, and the best way to exploit it is to wait on the spot for its sudden appearance. One phoretic fly that is not a kleptoparasite is the sphaerocerid *Acuminiseta pallidicornis* Villeneuve, which rides on the backs of giant (20 cm) millepedes in West Cameroon (Disney 1974, Roubaud 1916). It apparently breeds in millipede droppings and mounted flies wait for their hosts to defecate (riding phorids on the same hosts may also lay their eggs in the feces, Schmitz 1939, see also phoretic insects that develop in the dung of sloths, Waage & Montgomery 1976, Ratcliffe 1980, and macropodids, Norris 1991). Some species of Sphaeroceridae and Drosophilidae are phoretic on terrestrial crabs for similar reasons, but lay eggs on the hosts and have larvae which stay on the host and develop in the microbe-rich waste material that accumulates on the "felt glands" (Gomez 1977, Carson 1967).

Alternatively, the medium in which the larvae of phoretic flies develop is not just a byproduct of the larger animal, but a valuable resource that has been sequestered or produced by the host. For example, a tephritid, *Taeniostola limbata* Hendel, lays its eggs in the oviposition holes bored by large weevils into bamboos (Kovac & Azarae 1994). One or two individuals will spend hours on the elytra of the beetle waiting for it to complete its laborious chore, then hop off and be the first to lay their eggs. If the fly larvae compete with the beetle grub for food or space, then *T. limbata* could be categorized as a kleptoparasite. The two major forms of larval kleptoparasitism are reviewed below.

Kleptoparasites of Aculeate Hymenoptera. Nests of social Hymenoptera and food stores of solitary Hymenoptera support a wide variety of kleptoparasitic larval Diptera, although it is sometimes difficult to distinguish between kleptoparasitic and scavenging habits among the former group. In some cases the association between the larval kleptoparasite and its host is quite close, as in the phorid *Cataclinusa pachycondylae* (Brues), which attaches itself to a host ant larva and steals food as its associate is fed masticated prey (Wheeler 1910). Other myrmecophilous phorid larvae are highly specialized, though not phoretic, and are groomed and fed by worker ants (Disney 1994).

Food stores of solitary aculeates are an obvious target for theft, and are attacked by a wide variety of specialized kleptoparasites. Both pollen-storing (Moradeshaghi & Bohart 1968) and flesh-storing species are robbed by larval miltogrammine Sarcophagidae. Adult miltogrammines are usually closely associated with the nesting area rather than the adult host itself, typically mating nearby and larvipositing in or around the entrance to the host nest. Some miltogrammines, especially the genus *Senotainia*, are more closely associated with the adult host, and have earned the name

"satellite flies" for their habit of tracking foraging adult sphecid wasps. Satellite flies deposit larvae in the nest or on prey as it is being carried into the nest (Evans 1966). Other Miltogramminae (*Ptychoneura* spp.) deposit fully incubated eggs directly on the host (Day & Smith 1981). Larval kleptoparasites of solitary aculeates are also found in the Bombyliidae and Chloropidae. Species of the bombyliid genus *Lepidophora* develop on the provisions of Vespidae and Sphecidae (Hull 1973), and the chloropid *Lasiopleura grisea* Malloch has been reared from the nests of the sphecid *Bembix cameroni* (Evans 1966).

Kleptoparasites of Dung-feeding Scarabs. A diverse group of kleptoparasitic Sphaeroceridae ride upon dung-feeding scarabs in order to reach oviposition sites (Chobaut 1896, Roubaud 1916, Villeneuve 1916, Fletcher 1909, Collin 1910, Moulton 1880, Knab 1915, Steyskal 1971, Fig. 2). In fact, the term "phoresy" was coined by Lesne (1896) to describe the behavior of the sphaerocerid *Ceroptera rufitarsis* Meigen riding on the "Sacred Scarab", *Scarabaeus sacer* L., in the sand dunes behind Algerian holiday beaches.

The clumped and ephemeral nature of dung and its often substantial food value (e.g., human feces are ~50% bacteria) can result in fierce competition among its consumers (Wilson 1971, Bartholomew & Heinrich 1979, Rabkin & Silverman 1979). A number of scarabs avoid such competition by burying caches of feces, both for their own consumption and as food for their larvae. Burials can occur either near the dropping, in which case the burrow may be relatively deep (e.g, *Phanaeus* spp.), or the feces can be shaped into a ball and rolled a considerable distance before being buried in a shallow burrow (e.g., *Canthon* spp.). In one Florida cattle pasture the feces cached by scarabs contained 11× fewer Nematocera, 7× fewer nonphoretic Sphaeroceridae, 4× fewer Cyclorrhapha, and 6× fewer predaceous insects than the above ground "pats" from which the buried dung had been detached (Sivinski 1983).

These less-contested caches are in turn exploited by kleptoparasites, which include not only flies but even tiny phoretic Scarabaeidae (Hammond 1976). Of course by sidestepping many small competitors the kleptoparasite is confronted with a single very large one, the beetle itself. But scarabs are messy eaters, and there is often a good deal left over, some smeared into the burrow walls. In the laboratory, the numbers of offspring of the kleptoparasite *Norrbomia frigipennis* (Spuler) developing in food caches of the ball-rolling scarab *Canthon pilularius* (L.) decreased 63% when the beetle was also included (Sivinski 1983, the extraordinary range of adult size in this species may reflect some broods facing exceptional nutritional difficulties). A thief fly might also have the opportunity to oviposit in a more long lasting "brood ball" containing the offspring of the beetle, although there are special problems associated with this situation including parent beetles removing foreign insects from the dung mass and the encasing of the feces in soil (e.g. Halfter 1997).

In Florida there is a number of kleptoparasitic sphaerocerids, including a species with reduced eyes, *Ceroptera sivinskii* Marshall, that principally attaches itself to beetles that start their burrows under feces, and a mostly crepuscular species, *Norrbomia singularis* (Spuler) (Sivinski 1983, Marshall 1983). There is even a species, *Ceroptera longicauda* Marshall, that exploits a "non-dung beetle", the scarab *Peltotrupis profundus* Howden which may bury decaying organic material or fungus (J. S., personal observation., see also the Australian scarab *Cephalodesmius armiger* Westwood which constructs brood masses from green leaves and its kleptoparasite *Biroina myrmecophila* (Knab & Malloch) [Montieth and Storey 1981]). The most abundant kleptoparasitic species in north Florida is the previously mentioned *Norrbomia frigipennis,* an attractive black fly with white wings and red eyes. It rides upon a broad range of "rolling" and "burying" scarab hosts (Sivinski 1983), although in the laboratory it has a slight preference for species of *Phanaeus*, which are among the larger of the available dung beetles (Petersson & Sivinski 1997).

Fig. 2. The phoretic sphaerocerid *Ceroptera longicaudata* upon the geotrupin *Mycotrupes gaigei* Olson & Hubbell. This fly rides on species of the related genus *Peltotrupes*. The burrows of these beetles are often very deep (sometimes 3>m), and at such depths their brood materials are likely to be safe from most other thieves and predators. (Photograph by S. M.)

The seasonal pattern of *N. frigipennis* abundance in north Florida may reflect the advantages of kleptoparasitism (Sivinski 1983). In late winter and early spring the community of Diptera developing in bovine dung undergoes a change. Nematocera, particularly Sciaridae and nonphoretic sphaerocerids, become less numerous while calypterates, principally Sarcophagidae, increase rapidly. It may be that the large, quick growing flesh flies competitively exclude most sphaerocerids. *Norrbomia frigipennis* is an exception to the trend, its numbers continue to expand, perhaps because it avoids contact with calypterates by ovipositing in scarab dung stores.

While kleptoparasitism appears to be a means of avoiding competition and unfavorable environmental conditions the benefits of phoresy are more obscure, especially in light of the considerable costs in terms of time. Mature adult sphaerocerids of many species, including *N. frigipennis*, ride their hosts underground and once buried cannot leave until they accompany the departing beetle to the surface (Sivinski 1983). Newly eclosed adults are prodigious diggers, but this does not seem to be the case once their exoskeletons harden. A fly can expect to spend a day, and perhaps several days or more, buried alive with its host, a sizable portion of a ~10-12 day life span.

Why stay with a particular scarab? Why not go from beetle to beetle depositing eggs in the dung that each is rolling or pushing into its burrow? In fact some kleptoparasites, *Ceroptera longiseta* (Villeneuve) and *C. nasuta* (Villeneuve) from central Africa, ride beetles as they move feces but oviposit as the balls are being buried and do not get trapped beneath the surface (Roubaud 1916). An unidentified Florida *Pterogramma* spp. follows scarabs rather than rides and appears to oviposit on dung as it

disappears underground (Sivinski 1983, see a Mexican species with similar following habits in Halffter & Matthews 1966). Why don't other kleptoparasites subscribe to this seemingly more sensible practice? Perhaps the eggs could be damaged as the feces are manipulated and packed into a burrow. Whatever the reason, *N. frigipennis* were only reared from dung caches that had been buried for at least 4 hours (Sivinski 1983).

When oviposition is best accomplished underground, flies need to stay close to a host that might dig out of sight at any second. Given the need to stay close, it is probably cheaper to ride the beetle than to walk behind it. It may also be safer to be attached to one of the larger and least vulnerable animals in the dung-feeding community. Predators abound around droppings, and some, such as the reduviid *Apiomerus crassipes* Fab., even appear to follow fecal odors in order to locate hunting grounds (Sivinski 1983; an African *Ceroptera* sp. rides underneath scarabs, suggesting a predator that can glean flies from a beetles dorsum, [Hanstrom 1955-67]). When flies are committed to a beetle it might further be prudent to stay with it as it flies from one dropping to another and so avoid the risk of not finding a host in a new location. *Norrbomia frigipennis* clings to flying scarabs, particularly species of *Phanaeus,* and up to a dozen or so flies can be seen packed into forward-facing ranks on the great and glittering prothorasic shield of a male *P. vindex* MacLeay as it buzzes by (see Vulinec 1997).

The Distribution of the Sexes in Flies Phoretic upon Predators and Dung Beetles

With a few revealing exceptions to be discussed later, the flies from all six families that are found upon predators or their prey are females (Sivinski 1985 and cit.; see however records for occasional male milichiids and lonchaeids in Eisner et al. 1991, and Dobson 1992). Presumably it is only females that require proteins, probably for egg production. But why don't males take advantage of female concentrations in order to find mates?

There are two general reasons why males might not be found in the same places as females (Thornhill & Alcock 1983). The first is that the females in certain locations have no sexual value. Suppose females of a particular species control copulations and mate only once. As a consequence males will search for virgins and tend to accumulate at emergence sites (or swarm sites; see Sivinski & Petersson 1997). By the time females are feeding or ovipositing they are likely to have already been inseminated, making it too late for males to look for sexual encounters in such spots. Mosquitoes provide a commonly encountered example of this phenomenon (Sivinski 1984). For the most part the bloodthirsty legions hovering about our heads and the flanks of cattle are composed entirely of females that have copulated previously, over small water-filled containers, pond margins, or swarm markers.

The second reason males may not search for females at a feeding site is that the resources are too abundant relative to the numbers of females. The low probability that a female will be at any particular spot makes foraging among sites, or loitering at a site, an expensive and time-consuming business. Some of the predator kleptoparasites seem to be very rare (see Sivinski 1985), and males could search among spiders or wait around a particular web for a very long and unrequited time before encountering a mate. Presumably, males would instead either concentrate their efforts around oviposition/emergence sites (probably decaying vegetation and seeds or grass tillers in the case of milichiids and chloropids, Ferrar 1987, Teskey et al. 1976) or participate in swarms or leks at "encounter-convention" sites (e.g. Parker 1978).

Which, if either, of these explanations accounts for seldom seen predator-kleptoparasite males is unknown, although the second, an unfavorable ratio of females to feeding sites, has a shred of circumstantial support. Matings on the bodies of predators and on their prey have been observed in two species of the chloropid genus *Olcella*

Fig. 3. An unusual sight, mating by the predator-kleptoparasite *Olcella quadrivittata* on prey held by its robber fly host. Typically, only females of such flies are found on or near the "host", and mating in association with a predator has only been observed in a few species of this genus. (Photograph by S. M.).

(Sivinski 1985, Marshall 1998, Fig. 3), both of which appear to be "waiters", i.e., they instantly appear on prey captured by spiders, robber flies, and possibly mantids. Both species can be unusually abundant at certain times and places. Perhaps high populations of females make it profitable for males to also wait around large predators. A higher female to host predator ratio means males have a reasonable expectation that females will show up at any particular host.

Among dung beetle-kleptoparasites everything is different. Males are as common as females on the backs of scarabs and flies engage in repeated copulations both above and below ground (Fig. 4). In laboratory arenas female *N. frigipennis* spent an average of 25% of their time repeatedly mating (in one case 70%, Sivinski 1983). It is the potential for multiple inseminations that is probably responsible for males following a relatively few females underground and consequently diminishing their chances of finding new mates. Typically, sperm from the last of a series of inseminations are used to fertilize most of the eggs a female lays (Parker 1970). Because oviposition occurs underground, the opportunity for the valuable last mating is beneath the surface as well.

In some instances, large numbers of flies and hosts might even allow both male and female flies to choose a mount on the basis of its sexual opportunities, i.e., the prospective mates and sexual rivals already on board the scarab. These kinds of choices would be reflected in the composition of beetle-back groups. Large and small groups of *N. frigipennis* on the ball-rolling scarab *C. pilularis* tend to have female-biased sex ratios (Sivinski 1982, Petersson & Sivinski unpub.). When the patterns of male and female abundances are compared to random distributions, the sex ratio biases appear to be due largely to males being less numerous than expected in small groups and females being much more common in large groups than chance would predict. It is plausible that a male would avoid unoccupied beetles or those with a few other flies

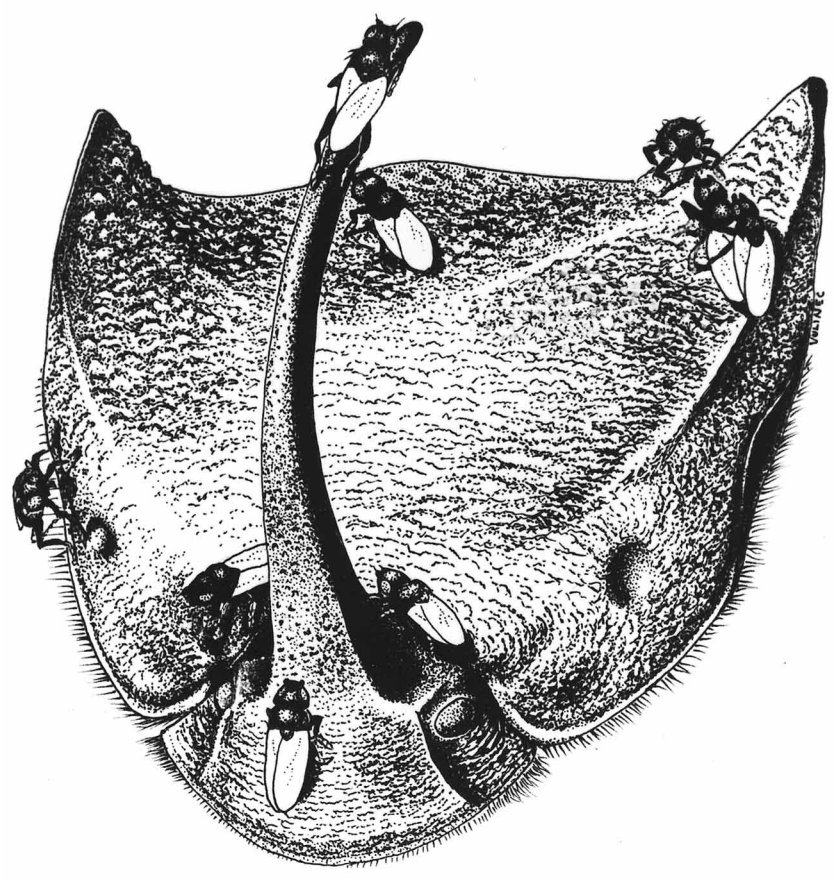

Fig. 4. A group of *Norrbomia frigipennis* wait and mate upon the head and prothoracic shield of a male of the dung feeding scarab *Phanaeus vindex*. This is a common association in Florida, and the fly can be found riding beetles as they walk on the surface, burrow in the ground, and fly through the air. (Drawing by Kevina Vulinec).

aboard, otherwise he could wind up underground without a sexual partner. On the other hand, a female, particularly a mated female, might benefit from belonging to a small group because her offspring would face fewer competitors. But, if this is the case, why are females "over represented" in large groups? Perhaps there are sexual reasons. Either virgin females or females seeking superior mates would have a greater pool of sexual partners to sample in larger groups.

There is some support for the notion that some mated females do not prefer large groups (Petersson& Sivinski, unpub.). In a laboratory experiment where mated and virgin *N. frigipennis* of both sexes had a choice of mounting one of a pair of beetles with different numbers of freeze-dried conspecifics glued to the elytra, the only significant response was that mated females avoided large groups. In keeping with the argument that virgins would prefer larger groups, there was a significant positive

correlation between the proportion of virgin females in field collected groups and the size of the groups.

The notion that at least some females prefer a large sample of prospective mates supposes that they can choose from what is available or, in the absence of choice, that the "fittest" males on beetle-back have greater access to females. There is no obvious male courtship in *N. frigipennis* that would serve to advertise desirable characteristics. However, large size is an easily perceived trait that might indicate "genetic quality" or be advantageous in competitions between males. If so, it might also be a quality females would like to see inherited by their sons. In the laboratory where large and small males were placed with a single female, the proportion of large male encounters with females that lead to copulation was significantly higher than those involving small males (Sivinski 1984). It could be that females preferred large males and were more likely to acquiesce, or that large males were better able to force themselves onto uncooperative females. It also appears that male-male competition filters out the smaller males and makes them less likely to contact females. Small males are just as liable as large to initiate an interaction with another male, but they are much less likely than large males to encounter females. This suggests that the vicinities around females are "controlled" by large males who exclude smaller rivals. There is also a negative correlation between male size and the proportion of time actually spent on scarabs, as opposed to following behind them. Again, perhaps larger males are able to dispossess the smaller and keep them from locating mates.

Conclusion: Kleptoparasite Diversity

Flies are the master thieves of their world. Take for example the predictably located and exposed food-treasures suspended in a spider's web. Only the formidable owner and her entangling snare stand in the way of a surfeit of protein, and a number of bold arthropods take the risk for the reward. Among these are other spiders (e.g. Vollrath 1979), a mirid bug (Davis and Russell 1969), scorpionflies (Thornhill 1975), damselflies (Vollrath 1977), fireflies (Provonsha 1998), and even Lepidoptera larvae (Robinson 1978). But no other kleptoparasitic group, of insects at least, seems to be as abundant, or as diverse, as the Diptera. Much the same may be said for the insects found infesting the dung-stores of scarabs, and flies, particularly species of Phoridae, are a major component of the "*food-sharing*" fauna living in the underground nests of social insects (e.g. Wilson 1971; Disney & Kistner 1997).

However, there appear to be differences in diversity within these various kleptoparasite guilds. For example, the symbionts of dung beetles are largely (entirely?) Sphaeroceridae, although flies of this family are certainly not the only Diptera found near feces. The only known exception in north Florida is a sort of proto-kleptoparasitism practiced by the sarcophagid *Ravinia derelicta* (Walker) which preferentially larviposits in the dung balls of *Canthon pilularis* and in the soft, moist "work faces" on the feces where the beetles labor (Sivinski 1983). On the other hand, the dipteran kleptoparasites of spiders include species of Milichiidae, Chloropidae, Lonchaeidae, Phoridae, Empididae, and even Nematocera in the families Ceratopogonidae and Cecidomyiidae.

The barriers these different types of hosts place around their resources must differ in permeability to the flies that prowl outside them. Perhaps the subterranean nature of food stores in scarabs and ground-dwelling social insects presents a more formidable problem to the typical fly than the open air exposure of prey held in jaws or suspended in a web. The above ground nests of social wasps and bees may be even more impenetrable. Only the tiny bee louse, apparently alone among all of the sugar-seeking flies, has been able to exploit the riches of the honeybee.

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DEDICATION OF 1998 ARMYWORM SYMPOSIUM TO DR.BILLY RAY WISEMAN: PLANT RESISTANCE EXPERT

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ABSTRACT

Dr. Billy Ray Wiseman of et al. the United States Department of Agriculture, Agricultural Research Service, Insect Biology & Population Management Research Laboratory at Tifton, GA has authored or co-authored over 300 refereed scientific papers, book chapters, review papers and/or bibliographies dealing primarily with plant resistance to insect pests, especially lepidopterans attacking corn and sorghum. He and his co-workers have released over 80 germplasm lines of corn and sorghum resistant to the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), corn earworm, *Helicoverpa zea* (Boddie), and sorghum midge, *Contarinia sorghicola* (Coquillett), during his 31 years of federal service. Additionally, his field and laboratory techniques are widely used by researchers around the world.

Key Words: Fall armyworm, host plant resistance

RESUMEN

El Dr. Billy Ray Wiseman del Departamento de Agricultura de los Estados Unidos, Servicio de la Investigación Agrícola, Laboratorio de Investigaciones sobre la Biología del Insecto y el Manejo de Poblaciones, en Tifton, GA, ha producido o co-producido más de 300 escritos científicos arbitrados, capítulos de libros, reseñas y/o bibliografías que tratan principalmente sobre resistencia de las plantas a insectos plaga, especialmente insectos lepidópteros que atacan al maíz y al sorgo. Durante sus 31 años de servicio federal, él y sus colegas han plantado más de 80 líneas de germoplasma de maíz y sorgo que son resistentes al gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), al gusano del elote del maíz, *Helicoverpa zea* (Boddie), y a la mosquita del sorgo, *Contarinia sorghicola* (Coquillett). Adicionalmente, sus técnicas de campo y de laboratorio son utilizadas por investigadores en todo el mundo.

The 1998 Armyworm Symposium is dedicated to Dr. Billy Ray Wiseman (Fig. 1), for his many contributions to plant resistance, especially to corn and sorghum insect pests.

Billy was born March 28, 1937, as the fourth child of Calvin and Beulah Wiseman of Sudan, TX. He was raised on a farm and received all of his secondary education from the Sudan school system. His high school activities included sports (football and basketball) and being a member and officer of the Future Farmers of America. Billy's senior year was highlighted when named valedictorian of his class.

After graduation, he entered Arlington State College and finished his undergraduate B.S. Degree in Agricultural Education at Texas Tech in 1959. His graduate degrees were earned from Kansas State University under the direction of Dr. Reginald Painter, the father of host plant resistance to insects. In 1961, Billy received a M.S.

Fig. 1. Dr. Billy Ray Wiseman: Plant Resistance Expert.

Degree with a double major in Entomology and Horticulture. At this point, he obtained a direct commission as an officer in the U.S. Army Medical Service Corp and served about three years in the Womack Army Hospital's Preventive Medicine Section at Fort Bragg, NC. It was during this period that he met an Army nurse named Gladys Mary Striegler from Hye, TX. A romance began that led to their marriage on November 2, 1963.

After completion of military duty, Billy and Gladys returned to Kansas State University where he pursued a doctoral degree in Entomology. October 3, 1966 was a special day in Billy's life. On this day, Billy passed his final Ph.D. orals and Gladys had their first child, William Samuel Wiseman II. Two years later they became the proud parents of a daughter, Amy Lucretia.

Billy began his professional career in 1967 at Stillwater, OK with ARS-USDA under the supervision of entomologist, Harvey Chada. After a year, he transferred to what was then called the Southern Grain Insects Research Laboratory (ARS-USDA) at Tifton, GA where he has served as a research entomologist for the last 31 years. Billy has worked under the directorship of H. C. Cox, Alton Sparks and Charlie Rogers.

During his career, he has authored or co-authored over 300 refereed scientific papers, including 13 book chapters, review papers or bibliographies (see list of fall armyworm papers). His ability to work effectively with his colleagues is evidenced by coauthoring papers with over 110 scientists. Additionally, he and his co-workers have released 80 corn and sorghum germplasm lines having resistance to either the fall armyworm (*Spodoptera frugiperda* (J. E. Smith)], corn earworm [*Helicoverpa zea* (Boddie)], or sorghum midge [*Contarinia sorghicola*]. Billy has continuously transferred useful technologies to his colleagues in the areas of field screening of corn and sorghum for resistance to insect pests, bioassaying for antibiotic plant compounds in the laboratory, and determining mechanisms and bases of resistance. Also, he was a member of the team that discovered the chemical compound, maysin, as the major antibiotic factor in silks of some corn genotypes to the fall armyworm and corn earworm.

Along with his research duties, Billy found time to teach two courses in plant resistance at the University of Florida. For 17 years, he traveled to Gainesville on the weekends to teach students principles of plant resistance and methodologies used in developing insect resistance plants and in understanding the mechanisms and bases of resistance. During these years he also served as graduate advisor for two Ph.D. students and as a committee member for four other students.

Incredibly, Billy, a highly productive bench scientist and part-time teacher, also found the time and energy to provide leadership and service to regional and national entomological societies, corn and sorghum groups and plant resistance workshops and symposiums. He chaired the Entomological Society of America's (ESA) membership committee, served as president of the Southeastern Branch of ESA and as the Branch's representative on the ESA Governing Board. He was one of the organizers of the Southeastern Branch's Armyworm Symposium. Recently, Billy was one of three organizers of two international symposiums on the development of corn resistant to insects held at CIMMYT in Mexico. Proceedings of these symposia provide present and future researchers with a foundation of information on entomological and plant breeding techniques used to develop resistant corn and recent advances and utilization of resistant germplasm. Anyone that has worked with Billy on a conference, symposium or committee soon realizes how efficient and prompt this man is in completing tasks assigned him.

Through the years, Billy's peers have recognized him as an outstanding scientist and cooperator, as an effective entomology leader and as an expert in plant resistance to insects. The following are comments from his peers concerning their thoughts of Billy.

Dr. Wiseman's contributions to fall armyworm control are well-known through his many scientific reports and resistant germplasm releases. Without question these accomplishments have earned him the deserved reputation of host plant resistance expert. Dr. Wiseman's colleagues know him as a scientist who, whether a leader or member of a team, will always give more than is expected to the research effort. Perhaps one of his greatest contributions to armyworm research is how he always inspires and assists other researchers to do their best. From the beginning of my career in entomology, and especially during the past several years, I have benefited greatly from his mentorship.

I have been associated with Billy Wiseman for over thirty years. He has been a gentleman, an outstanding teacher and researcher. He understood host plant resistance when I first met him, but since then he has added much scientific information to our research base. Billy has studied several crops and several insects and their interactions. He is an authority for host plant resistance with corn and the corn earworm and fall armyworm in the field and in the laboratory. Dr. Wiseman is one of the most cooperative scientists I know. He has freely shared his research information, his talent for presenting it, as well as the research tools he has developed. Billy's nature motivates scientists to work cooperatively and collaboratively and makes the world a better place to live as he has shared his talents around the world.

Billy Wiseman is a hard worker, dedicated to increasing our knowledge and understanding of plant resistance to insects. You can count on his use of appropriate experimental design, statistical analysis and interpretation of the data. His interests extend beyond the mechanisms of plant resistance to interactions between plant resistance and other control strategies such as the use of insect pathogens, parasitoids and predators and their potential use in integrated pest management. His love of teaching has helped to keep him current on developments in his area of specialty and made him willing to take the time to explain things to cooperators who come from a different specialty. Billy's enthusiasm and cooperative nature have made it a joy to work with him to investigate interactions between plant resistance and insect pathogens of fall armyworm and corn earworm.

Is Billy Wiseman an expert in host plant resistance? You bet he is! He has spent his career looking at how those crawling creatures interact with so many important crop plants. This does, however, bring up a very important question: Why is he so good at what he does? I think I know the answer. If a DNA analysis of Billy were to be run, the results would probably show a positive match with several corn and sorghum pests. This would help to explain why he knows those pests so well. He is also a positive match with his colleagues. I have cooperated with Billy on many research projects over the past several years. I know him to be an excellent researcher and he is totally unselfish when it comes to sharing data and publishing results. Billy is top notch.

I have known Dr. Billy Wiseman for just a little over 10 years. Yet, it seems like I have known him twice as long. From my perspective, these were all positive experiences. During this time, I have had a good opportunity to see some of his contributions toward the betterment of humanity. He has a continual willingness to aid others without looking for recognition—a true team player. When I worked at the IBPMRL in Tifton, although we were on separate projects, Billy did not hesitate to offer suggestions from his years of experiences. I appreciated his professionalism and quickly recognized and respected his depth of knowledge of host plant resistance, and other subjects. I have published with Billy and because I value his frankness and expertise, I have also asked him to review some of my manuscripts. Not only do I much appreciate his helpful comments, but he always returns the reviews so quickly! Thanks for helping my career along in so many ways. Traveling by car to distant meetings provides a

good opportunity to foster a closer bond with colleagues. It was always a good pleasure to travel with Billy. I recall his long patience and his much agreeableness. Also, I have had the pleasure to become acquainted with his family; he is obviously a caring husband and father. It has been a good pleasure to share part of the highway of life with Billy. Thanks for being such a good role model.

It has been both an extreme privilege and a great benefit to me to have had the opportunity to be both a cooperator and friend of Bill Wiseman. I cannot imagine how lacking my career and my life would have been had I not known him.

Not only do his peers consider him an expert in plant resistance to insects but some have nominated him for special recognition. Some honors that Billy has received are: Fellow of the Entomological Society of America; J. E. Brussart Award (Southeastern Branch of ESA); Achievement Award from the Florida Entomological Society for Significant Contributions to the Science and Technology of Plant Resistance to Insects, and Achievement Award of Excellence as Senior Scientist, USDA-ARS, South Atlantic Area.

Dr. Billy R. Wiseman has distinguished himself as an expert in plant resistance to insects, a highly productive scientist, a mentor to students and colleagues, an extra special collaborator, a leader in entomological activities and a true gentleman. Therefore, it is our pleasure and honor to dedicate the 1998 Armyworm Symposium to Dr. Billy Ray Wiseman—Plant Resistance Expert.

A Listing of B. R. Wiseman's Fall Armyworm Publications (1966-1997)

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BACILLUS THURINGIENSIS FOR USE AGAINST ARMYWORM, *PSEUDALETIA UNIPUNCTA* (LEPIDOPTERA: NOCTUIDAE), ON WHEAT

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ABSTRACT

Spray table tests with *Bacillus thuringiensis* (Javelin WG) on wheat leaves against armyworm, *Pseudaletia unipuncta* (Haworth), showed that 1st and 3rd instars had LC_{50}s of 0.09 and 0.55 kg per ha, respectively, 7 d after treatment. Wheat sprayed in the field in 1995 with Javelin WG at rates of 0, 0.28, 0.56, 1.12, and 2.24 kg per ha resulted in 82% and 62% 1st instar mortality 7 d after feeding on treated flag and middle leaves, respectively, at the highest rate. In 1996, the test was repeated and mortality was higher, with 98, 97, and 92.9% 1st instar mortality 7 d after feeding on treated flag, middle, and bottom leaves, respectively, at the highest rate. Third instars were less susceptible, with 89, 81, and 76% mortality 7 d after treatment at the highest rate on flag, middle, and bottom leaves, respectively. Leaf position had little effect on spray droplet numbers or mortality of larvae fed treated leaves, indicating that spray distribution was relatively even throughout the wheat canopy.

Key Words: biological control, microbial control, spray distribution

RESUMEN

Pruebas de atomización en hojas de trigo con rocío de *Bacillus thuringiensis* (Javelin WG) contra el gusano cortador, *Pseudaletia unipuncta* (Haworth), demostraron que los 1er y 3er estadíos tenían, respectivamente, los LC_{50}s de 0.09 y 0.55 kg por ha, 7 días después de las aplicaciones. Pruebas de campo llevadas a cabo 1995 en trigo rociado con Javeline WG con dosis de 0, 0.28, 0.56, 1.12, y 2.24 kg por ha resultaron en una mortalidad de 82% y 62% en el primer estadío 7 días después de alimentarse en las hojas terminales y medias, respectivamente, tratadas con la dosis más elevadas. En 1996, se repitieron las pruebas obteniendo una mortalidad más alta, con el 98, 97, y 92.9% de mortalidad del primer estadío después de 7 días de alimentarse en las hojas terminales, medias y basales, respectivamente, con las dosis mas elevadas. El tercer estadío fue menos susceptible, con una mortalidad de 89, 81, y 76% 7 días después del tratamiento con las dosis más elevadas en hojas terminales, medias, y basales, respectivamente. La posición de la hoja tuvo un efecto pequeño en el numero de gotas de rocío o en la mortalidad de las larvas que se alimenaron en hojas tratadas, indicando que la distribución del rocío fue relativamente uniforme a traves de toda la corona del trigo.

The armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) is a pest of wheat in the US, with large acreages of heading wheat requiring treatment in some years (Breeland 1958, Guppy 1961, Clark et al. 1994). Biological insecticides are not registered in Arkansas for use on wheat. Three baculoviruses and six *B. thuringiensis* products were effective against *P. unipuncta* in laboratory assays on diet but were ineffective at a range of rates in field trials on commercial wheat (Steinkraus & Young 1994, Young & Steinkraus 1996). In those previous field trials, 4 and 7 d after treatment there were no significant differences between numbers of larvae in control and treatment plots. The main objective of this investigation was to determine if spray coverage significantly influenced performance of *B. thuringiensis* on wheat. This was tested by determining spray deposition throughout the wheat canopy with water sensitive cards and by feeding armyworm larvae treated leaves from three leaf positions.

MATERIALS AND METHODS

Gravid female armyworm moths were collected each year in early spring from an ultraviolet light trap. Moths were held in aquaria and fed a mixture of honey, beer, and water (9:7:14). Eggs were collected daily on folded wax paper fans attached to the sides of the aquaria and held at 29°C until hatch. Larvae were placed on semisynthetic diet (Burton 1969) in 270 ml wax-coated paper cups at 29°C until ready for use in the tests as either 1 - d old 1st instars or 3rd instars.

Wheat, ('Cardinal'), was planted 17 November 1995 and 15 September 1996 on the Arkansas Agricultural Research and Extension Center in Fayetteville, AR and grown according to commercial practices for the area.

Spray Table Test

On 15 May 1995, when heading wheat was in the soft dough stage [stage 7.7 of Zadoks et al. (1974)], middle leaves were cut from plants, placed in plastic bags to minimize drying, and brought to the laboratory. The leaves were placed upper surface up on a 91 by 91 cm board for spraying. Treatments were Javelin WG (Sandoz Crop Protection Corpora-

tion, Des Plaines, IL) at 0, 0.56, 1.12, and 2.24 kg per ha. Treatments were applied using a boom-type sprayer, with two TX-6 nozzles spaced 50.8 cm apart, at a pressure of 40 psi and a spray table with a speed of 4.8 km per h. The sprayed leaves were briefly air dried, then single leaves were placed individually into 28-ml plastic rearing cups. Either a 1st instar or 3rd instar was placed on the leaf, the cup capped and held at 29°C for 3 d. Treatments consisted of 25 larvae per instar per rate and the test was replicated four times for both 1st and 3rd instars. All larvae on treated leaves in cups were placed into sealed clear plastic containers that contained a wetted paper towel to minimize desiccation of the wheat leaves. After 3 d mortality was recorded and surviving larvae were transferred to semi-synthetic diet in individual 28-ml rearing cups. Larvae were held on diet until the 7th day after treatment and mortality was again recorded. Data were corrected for control mortality by Abbott's formula (Abbott 1925). The lethal concentration mortality response was estimated by the probit response (SAS Institute 1990). Failure of 95% confidence limits to overlap was used as a criterion for significant difference.

Field Tests

Field tests were conducted in 1995 and 1996 to determine penetration of *B. thuringiensis* spray droplets into the wheat canopy, specifically droplet numbers hitting flag, middle and bottom leaves. In all field tests, wheat was heading [stages 4.5-7.7 of Zadoks et al. (1974)]. Each test was a randomized complete block design, replicated four times over time (one replicate per day). Plots were 2.1 × 9.2 m with 1.8 m borders around plots. Treatment rates were Javelin WG at 0, 0.28, 0.56, 1.12, and 2.24 kg per ha. Treatments were applied using a backpack CO_2 sprayer with a 2-row boom and two TX-6 nozzles per row set 0.5 m apart calibrated to deliver 95 l per ha at 4.8 km per h. After the spray dried, 25 flag, middle, and bottom leaves were collected at random from each treatment, placed in plastic bags and transported to the laboratory. Assay of Javelin WG on these leaves was with 1st and 3rd instars as described in the spray table test above. Field tests were made on the following dates: 1st instars were tested 24-27 May 1995, 3rd instars were tested 14-17 May 1996 and 1st instars 20-23 May 1996. Mortality data were transformed by arcsin squareroot, analyzed with ANOVA, and means separated by LSD ($P < 0.05$). Pearson correlation coefficients were determined for treatment rate correlation with mortality by day and leaf position (SAS Institute, 1990).

Water sensitive paper cards (Ciba-Geigy, Basle, Switzerland), 52 × 76 mm, were placed in the wheat field to monitor spray distribution at different heights of the wheat canopy. Card placement heights were based on the mean heights of flag, middle and bottom leaves on 25 randomly chosen plants in each year. Cards were held horizontally by metal, double-prong hair clips hot glued to wire flags placed in the plots. Each wire flag had cards at heights of 45, 28, and 14 cm from the ground in 1995 and 43, 23, and 13 cm in 1996. Two card-holding flags were randomly placed in each plot prior to spray application (2 cards per height per treatment per day). Cards were collected immediately after the spray dried and brought into the laboratory. Droplets were counted with a hand lens in four areas per card using a 0.25 cm^2 window placed at random on the water sensitive cards. Mean numbers of droplets per 0.25 cm^2 were determined for each leaf height and treatment. Means by rate within a leaf position and by leaf position within a rate were separated by LSD tests (SAS Institute 1990).

Results

Spray Table Test

The lethal concentration curve for Javelin WG-treated wheat leaves fed to 1st instars resulted in a LC_{50} after 3 d of 0.53 kg per ha (Table 1). Mortality was much higher

TABLE 1. DOSAGE MORTALITY CURVES (KG/HA) FOR *P. UNIPUNCTA* LARVAE ON JAVELIN WG-TREATED WHEAT LEAVES USING A SPRAY TABLE.

Instar	Days after treatment	LC_{50} (FI)	LC_{90} (FI)	Slope	Chi-square P>
1st	3	0.53 (0.43-0.65)	5.52 (3.26-13.45)	1.256	0.001
	7	0.09 (0.05-0.12)	0.71 (0.56-1.04)	1.392	0.001
3rd	3	1.70 (1.34-2.38)	11.01 (6.51-24.14)	1.580	0.001
	7	0.55 (0.47-0.63)	4.12 (2.85-6.94)	1.458	0.001

7 d after treatment when the LC_{50} was 0.09 kg per ha. Third instars were approximately 3 and 6 fold less susceptible than 1st instars after 3 and 7 d, respectively. These data show that *B. thuringiensis* killed small larvae on treated wheat leaves and has potential for *P. unipuncta* control on wheat when timed against small larvae. The LC_{50} rates after 7 d for 1st and 3rd instar were lower than rates recommended for control of some lepidopterous larval pests on other crops (Johnson and Jones 1996).

Field Tests

At the time of the field tests, plant heights from base of stem to top of head in 1995 and 1996 were 57.5 (3.1) and 56.7 (1.9) cm, respectively [means, (SE)]. Heights of the flag, middle, and bottom leaves were 45.3 (1.8), 27.5 (0.9), 13.9 (0.7) cm in 1995, and 43.2 (0.9), 22.9 (0.6), and 12.6 (0.5) cm in 1996, above the ground, respectively [means, (SE)]. The spray distribution data (Table 2) indicated, that in most cases within a leaf position, significantly more droplets were found on cards in the control treatment (water only) than in the Javelin WG treatments (Table 2). There was seldom any difference in droplet density within a test and leaf position in those treated with Javelin WG. While in most cases significantly fewer droplets were counted on the bottom leaves relative to numbers on the flag leaves, it appears that inadequate penetration of the wheat canopy by *B. thuringiensis* sprays was not the cause of lack of efficacy in the field tests of Steinkraus & Young (1994) and Young & Steinkraus (1996).

In the 1995 field test, 1st instar mortality increased significantly with increases in Javelin rate at 3 and 7 d for flag and middle leaf positions (Table 3). Bottom leaves were senescing at the time of the test and therefore, data for this leaf position were not used. There were no significant differences in mortality due to leaf positions within a day, again showing that Javelin WG coverage of flag and middle leaves was similar. Mortality after 3 d was low in all treatments with only 47 and 25% mortality at the highest rate on the top and middle leaf, respectively. While the mortality at the higher rates was significantly higher at 3 d than the control, it was never greater than 50%. Mortality was higher after 7 d with 82 and 62% dead at the highest rate on the flag and middle leaves, respectively. Although most rates did not show a significant difference in mortality with leaf height (flag or middle leaf), in most cases mortality was higher on the flag leaf.

In the 1996 test using 1st instars, there was a significant positive correlation between increased Javelin WG rate and mortality for each day and leaf position (Table 4). In all cases significantly more larvae were killed in the Javelin WG treatments than the controls. Larval mortality after 3 d was higher than in the 1995 test with mortality at the highest rate reaching approximately 70% at all three leaf positions. Mortality after 7 d was higher reaching approximately 90% at 1.12 kg per ha at all

TABLE 2. NUMBERS OF *B. THURINGIENSIS* SPRAY DROPLETS ON WATER SENSITIVE PAPER CARDS AT HEIGHTS OF FLAG, MIDDLE, AND BOTTOM WHEAT LEAVES IN 1995 AND 1996 FIELD TESTS.

Javelin rate[2] (kg/ha)	Mean (SE) # droplets (per 0.25 cm^2)[1]		
	Card locations		
	Flag leaf[3]	Middle leaf[4]	Bottom leaf[5]
	24-27 May 1995 Test		
0	66.9 (9.1) a A	60.8 (8.9) a A	40.4 (5.9) a B
0.28	37.0 (3.7) b A	33.4 (4.5) b A	21.4 (2.7) bc B
0.56	39.9 (3.8) b A	34.3 (2.9) b AB	30.9 (3.8) ab B
1.12	19.9 (3.1) c A	19.4 (3.2) c A	14.6 (2.3) c A
2.24	55.2 (5.5) a A	43.7 (3.7) b B	41.5 (4.4) a B
	14-17 May 1996 Test		
0	68.2 (10.7) a A	61.6 (12.6) a A	66.4 (14.9) a A
0.28	47.7 (4.8) b A	44.1 (2.9) b AB	32.7 (4.6) b B
0.56	39.0 (4.6) b A	28.5 (3.0) b AB	22.2 (1.5) b B
1.12	38.6 (9.2) b A	41.6 (6.9) b A	30.7 (4.6) b A
2.24	42.6 (6.8) b A	35.2 (5.6) b AB	25.1 (7.4) b B
	20-23 May 1996 Test		
0	72.0 (9.1) a A	57.9 (8.1) a A	55.8 (7.3) a A
0.28	51.4 (4.4) b A	42.6 (4.1) ab AB	37.9 (5.1) b B
0.56	55.9 (4.5) b A	48.9 (4.5) ab AB	41.5 (3.0) b B
1.12	43.0 (3.0) b A	37.8 (5.1) b A	25.4 (2.2) c B
2.24	52.5 (5.2) b A	39.4 (3.4) b B	41.8 (1.9) b B

[1]Means within a column followed by the same lower case letter or within a row followed by the same capital letter did not differ significantly (ANOVA, LSD).

[2]ANOVA statistics for rate: 24-27 May 1995 Test, 0 rate ($F = 4.05$; $df = 2$; $P = 0.0219$); 0.28 rate ($F = 7.93$; $df = 2$; $P = 0.0008$); 0.56 rate ($F = 2.56$; $df = 2$; $P = 0.0851$); 1.12 rate ($F = 1.36$; $df = 2$; $P = 0.2648$); 2.24 rate ($F = 4.65$; $df = 2$; $P = 0.0128$): 14-17 May 1996 Test, 0 rate ($F = 0.14$; $df = 2$; $P = 0.8686$); 0.28 rate ($F = 4.12$; $df = 2$; $P = 0.0412$), 0.56 rate ($F = 5.85$; $df = 2$; $P = 0.0154$), 1.12 rate ($F = 0.69$; $df = 2$; $P = 0.5311$); 2.24 rate ($F = 5.47$; $df = 2$; $P = 0.0188$): 20-23 May 1996 Test, 0 rate ($F = 2.06$; $df = 2$; $P = 0.1565$); 0.28 rate ($F = 2.82$; $df = 2$; $P = 0.0859$); 0.56 rate ($F = 3.35$; $df = 2$; $P = 0.0580$); 1.12 rate ($F = 5.65$; $df = 2$; $P = 0.0125$); 2.24 rate ($F = 8.10$; $df = 2$; $P = 0.0031$).

[3]ANOVA statistics for flag leaf card locations: 24-27 May 1995 test ($F = 12.05$, $df = 4$; $P = 0.0001$); 14-17 May 1996 test ($F = 3.05$; $df = 4$; $P = 0.0395$); 20-23 May 1996 Test ($F = 3.65$; $df = 4$; $P = 0.0148$).

[4]ANOVA statistics for middle leaf card locations: 24-27 May 1995 Test ($F = 10.04$; $df = 4$; $P = 0.0001$); 14-17 May 1996 Test ($F = 4.40$; $df = 4$; $P = 0.0103$); 20-23 May 1996 Test ($F = 2.31$; $df = 4$; $P = 0.0792$).

[5]ANOVA statistics for bottom leaf card locations: 24-27 May 1995 Test ($F = 8.57$; $df = 4$; $P = 0.0001$): 14-17 May 1996 Test ($F = 9.18$; $df = 4$; $P = 0.0002$); 20-23 May 1996 Test ($F = 6.55$: $df = 4$; $P = 0.0006$).

three leaf positions. There was no significant increase in mortality by increasing rate from 1.12 to 2.24 kg per ha. As in the 1995 test mortality was similar across leaf positions within a rate and day, showing that Javelin WG was penetrating the canopy and providing a similar dose to larvae feeding at all leaf positions.

In the 1996 test with 3rd instars, there was a significant positive increase in mortality with rate increases for all leaf positions and both days (Table 5). After 3 d, mor-

TABLE 3. PERCENTAGE MORTALITY OF 1ST INSTAR *P. UNIPUNCTA* FED *B. THURINGIENSIS*-TREATED FLAG AND MIDDLE WHEAT LEAVES TREATED IN THE FIELD (1995)[1].

Javelin WG rate (kg/ha)	Mean (SE) % Mortality[2]			
	3 day		7 day	
	Flag leaf	Middle leaf	Flag leaf	Middle leaf
0	3.0 (1.0) a A	4.0 (2.8) a A	11.0 (3.4) a A	8.0 (3.6) a A
0.28	29.0 (9.7) b A	23.0 (9.9) b A	56.0 (9.5) b A	41.0 (9.3) b A
0.56	32.0 (11.2) b A	17.0 (3.0) b A	52.0 (5.6) b A	45.0 (2.5) b A
1.12	33.0 (5.9) b A	34.0 (9.0) b A	66.0 (8.2) ab A	62.0 (6.8) b A
2.24	47.0 (3.4) b A	25.0 (5.5) b B	82.0 (5.8) c A	62.0 (12.9) b B
r for rate[3]	0.70	0.58	0.81	0.76
P > r	0.0006	0.0074	0.0001	0.0001

[1]Bottom leaves were senescing and were not suitable food for the larvae, so data is not presented.

[2]Within day means in a column (lowercase) or row (upper case) followed by the same letter are not significantly different (ANOVA, LSD, P > 0.05). ANOVA statistics for rate by leaf position within a day are as follows: df = 4 in all cases; 3 d statistics, (flag leaf F = 8.68, P = 0.0016), (middle leaf F = 4.34, P = 0.0211); 7 d statistics, (flag leaf F = 12.34, P = 0.0003), (middle leaf F = 7.90, P = 0.0023). ANOVA statistics for leaf position by day within a rate are as follows: df = 1 in all cases; 0 rate (3 day F = 0.01, P = 0.9314), (7 day F = 0.52, P = 0.5239); 0.28 rate (3 day F = 0.72, P = 0.4571) (7 day F = 5.74, P = 0.0963); 0.56 rate (3 day F = 0.85, P = 0.4249), (7 day F = 1.10, P = 0.3706); 1.12 rate (3 day F = 0.02, P = 0.8865), (7 day F = 0.29, P = 0.6269); 2.24 rate (3 day F = 21.8, P = 0.0185), (7 day F = 11.68, P = 0.0419).

[3]Pearson correlation coefficients (SAS 1990).

tality on flag leaves ranged from 17% at 0.28 kg per ha to 59% at 2.24 kg per ha. Mortality at 7 d was higher in most treatments and at the highest rate ranged from 76 to 89%. In no cases were there significant differences between mean mortalities due to leaf position within a day and rate.

DISCUSSION

The spray table test showed that greater than 50% mortality of 1st instar *P. unipuncta* could be achieved at recommended rates of *B. thuringiensis* on middle wheat leaves at either 3 or 7 days. Mortality occurred slowly and it was not possible to achieve a 50% mortality level at recommended rates of Javelin WG with 3rd instars at 3 d. Mortality levels in field-sprayed wheat tests were similar to those in the spray-table test indicating that spray distribution on leaves was satisfactory at all plant heights. Mortality in 3rd instars exposed to Javelin WG on wheat was slightly higher in the field test (Table 5) than would be expected based on the spray table data. For example, in the spray table test, even at 7 d, the LC_{90} was 4.12 kg per ha, whereas, in the field test, 89% were killed at 7 d at 2.24 kg per ha. This was unexpected since in the spray-table test mortality of 3rd instars had been several times less than that of 1st instar. Further testing will be required to explain these differences.

Results of the spray tests suggest that *B. thuringiensis* has potential to control *P. unipuncta* on wheat if treated as small larvae. These results are different from those of small-plot commercial wheat field tests in which several *B. thuringiensis* products failed to significantly reduce populations of *P. unipuncta* larvae (Steinkraus

TABLE 4. PERCENTAGE MORTALITY OF 1ST INSTAR *P. UNIPUNCTA* FED *B. THURINGIENSIS*-TREATED FLAG, MIDDLE, AND BOTTOM WHEAT LEAVES TREATED IN FIELD (1996).

Javelin rate (kg/ha)	Mean (SE) % Mortality[1]					
	3 day			7 day		
	flag leaf	middle leaf	bottom leaf	flag leaf	middle leaf	bottom leaf
0	1.0 (1.0) a A	1.0 (1.0) a A	3.0 (1.9) a A	3.0 (1.9) a A	2.0 (1.2) a A	12.0 (3.6) a B
0.28	26.2 (7.3) b A	22.1 (8.0) b A	54.3 (8.2) bc B	58.7 (8.4) b A	54.7 (6.9) b A	78.8 (11.6) bc B
0.56	54.0 (6.6) c A	50.0 (6.2) c A	49.7 (11.1) b A	88.0 (5.9) c A	84.0 (7.1) c A	74.2 (10.0) b A
1.12	56.0 (11.4) c A	65.0 (6.8) d A	66.7 (6.0) bc A	90.0 (4.8) c A	92.0 (1.6) cd A	89.9 (3.4) cd A
2.24	70.7 (11.5) c A	76.7 (6.0) e A	72.8 (5.9) c A	98.0 (1.2) c A	97.0 (1.9) d A	92.9 (1.9) d B
r for rate[2]	0.83	0.91	0.77	0.87	0.90	0.76
P > r	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[1]Within day means in a column (lower case; ANOVA, LSD) or row (upper case, ANOVA, LSD) followed by the same letter are not significantly different (P > 0.05). ANOVA statistics for rate by leaf position within a day are as follows: df = 4 in all cases; 3 d statistics, (flag leaf F = 19.28, P = 0.0001), (middle leaf F = 234.17, P = 0.0001), (bottom leaf F = 21.52, P = 0.0001); 7 d statistics, (flag leaf F = 66.53, P = 0.0001), (middle leaf F = 100.50, P = 0.0001), (bottom leaf F = 50.18, P = 0.0001). ANOVA statistics for leaf position by day within a rate are as follows: df = 2 in all cases; 0 rate (3 day F = 0.43, P = 0.6679), (7 day F = 5.95, P = 0.377); 0.28 rate (3 day F = 17.19, P = 0.0033); 0.56 rate (3 day F = 0.27, P = 0.7690), (7 day F = 2.77, P = 0.14060; 1.12 rate (3 day F = 0.42, P = 0.6766), (7 day F = 0.17, P = 0.8497); 2.24 rate (3 day F = 0.05, P = 0.9483), (7 day F = 5.71, P = 0.0409).

[2]Pearson correlation coefficient (SAS 1990).

TABLE 5. PERCENTAGE MORTALITY OF 3RD INSTAR *P. UNIPUNCTA* FED *B. THURINGIENSIS*-TREATED FLAG, MIDDLE, AND BOTTOM WHEAT LEAVES TREATED IN FIELD (1996).

Javelin rate (kg/ha)	Mean (SE) % Mortality[1]					
	3 day			7 day		
	flag leaf	middle leaf	bottom leaf	flag leaf	middle leaf	bottom leaf
0	6.0 (2.0) a	4.0 (2.3) a	10.0 (3.4) a	8.0 (3.2) a	9.0 (4.1) a	23.0 (5.2) a
0.28	17.0 (4.4) b	15.0 (4.7) ab	16.0 (4.9) ab	36.0 (5.9) b	32.0 (3.3) b	35.0 (10.4) ab
0.56	18.0 (3.5) b	20.2 (10.9) abc	21.0 (10.7) ab	50.0 (4.8) b	44.3 (13.2) bc	40.0 (14.1) ab
1.12	34.0 (6.6) c	37.0 (6.6) bc	35.0 (9.1) bc	56.0 (7.1) b	63.0 (10.7) cd	56.0 (10.3) bc
2.24	59.0 (7.4) d	44.0 (12.6) c	47.0 (13.8) c	89.0 (4.4) c	81.0 (4.1) d	76.0 (8.5) c
r for rate[2]	0.86	0.74	0.61	0.89	0.86	0.69
P > r	0.0001	0.0002	0.0043	0.0001	0.0001	0.0007

[1]Within day means in a column (lower case; ANOVA, LSD) followed by the same letter are not significantly different (P > 0.05). No significant differences were found for mortality by leaf position within a day and treatment (statistics not presented). ANOVA statistics for rate by leaf position within a day are as follows: df was 4 in all cases; 3 d statistics, (flag leaf F = 33.50, P = 0.0001), (middle leaf F = 5.75, P = 0.008), (bottom leaf F = 6.15, P = 0.0062); 7 d statistics, (flag leaf F = 22.57, P = 0.0001), (middle leaf F = 11.61, P = 0.0004), (bottom leaf F = 8.53, P = 0.0004).

[2]Pearson correlation coefficient (SAS 1990).

& Young 1994, Young & Steinkraus 1996). This may have been due to unexamined factors such as larval size during the tests. The field populations treated in the efficacy tests of Steinkraus & Young (1994) and Young & Steinkraus (1996) were of mixed ages with most 3rd instars or older. As the tests show, larger larvae are more difficult to kill than smaller larvae.

Another factor is deactivation of *B. thuringiensis* by sunlight. In assays of *B. thuringiensis* sprayed on other crops it had an activity half-life of approximately 2 days (Ali & Young 1993). In the tests reported here, leaves were collected immediately after spray deposits dried (usually less than 30 min). This minimized potential degradation of *B. thuringiensis* deposits by sunlight. In addition, once treated leaves were picked, brought to the laboratory, and placed in cups, test larvae fed throughout the 3 d leaf-exposure period on undegraded *B. thuringiensis.* In the field tests of Steinkraus & Young (1994) and Young & Steinkraus (1996), in spite of the fact that the *B. thuringiensis* products were applied in late afternoon to minimize UV degradation, the *B. thuringiensis* deposits would still have been exposed to several hours of sunlight before the *P. unipuncta* larvae fed. Armyworms typically feed at night and rest on the ground during the day. Thus, in field tests with feral *P. unipuncta* larvae *B. thuringiensis* residues would be increasingly degraded by sunlight each day throughout the duration of a test.

A third possible explanation of the failure of *B. thuringiensi*s products to reduce field armyworm populations, as reported by Steinkraus & Young (1994) and Young & Steinkraus (1996), could be movement of larvae between plots in field tests. It is possible that between the day of application, and assessments of larval populations 4 d later, armyworms moved between plots. If so, larvae counted in treated plots may have originated from outside treated areas and have been unexposed to *B. thuringiensis*. Such a situation could result in no significant differences in larval numbers between treated and control plots. Further tests are needed to fully explain the failure of *B. thuringiensis* to control *P. unipuncta* on heading wheat.

ENDNOTE

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LATE SEASON BEET ARMYWORM (LEPIDOPTERA: NOCTUIDAE) INFESTATIONS ON COTTON: DEFOLIATION, FRUIT DAMAGE, AND YIELD LOSS

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ABSTRACT

Field cage studies were conducted in 1996 and 1997 to measure the effects of late season beet armyworm, *Spodoptera exigua* (Hübner), infestations (0, 1, 3, and 6 egg masses per 5.1 m row) on defoliation, fruit damage, and yield of cotton. Significantly higher light penetration through the cotton canopy was observed in most infested plots compared with non-infested control plots. A trend for higher numbers of damaged fruiting forms (squares and bolls) with increases in egg mass density was observed. There were no significant differences in the number of damaged fruiting forms among treatments, however, plots infested with 1, 3, or 6 egg masses had 2.3, 2.4, and 3.3-fold more damaged fruiting forms than the control plots. In all infested plots, a significantly higher percentage of shed fruiting forms were damaged compared with the control plots in 1996. In 1997, only plots infested with 6 egg masses had a significantly higher percent of the cumulative fruiting forms damaged compared with the control plots. In both years, there were no significant differences in seed cotton yield among treatments.

Key words: *Gossypium hirsutum* L., Plant Damage, Yield Loss, *Spodoptera exigua*

RESUMEN

Se realizaron experimentos en 1996 y 1997 con jaulas en campos de algodón para medir el efecto de infestaciones de fin de temporada de *Spodoptera exigua* (Hübner), (con 0, 1, 3, y 6 masas de huevecillos por cada 5.1 m de hilera) en la defoliación, daño del fruto, y rendimiento del algodón. Se observó una cantidad significativamente más alta de penetración de luz a través del follaje del algodón en la mayoría de las parcelas infestadas en comparación con las parcelas no infestadas. Se observó una correlación positiva entre el número de órganos fructíferos (cuadros y bellotas) dañados y la densidad de masas de huevecillos. No se documentaron diferencias significativas en el número de frutos entre los tratamientos; sin embargo, parcelas infestadas con 1, 3, o 6 masas de huevecillos tenían 2.3, 2.4, y 3.3 veces más de frutos que las parcelas control. En todas las parcelas infestadas, un porcentaje significativamente más alto de frutos tirados estaba dañado por el gusano de *Spodoptera exigua* en comparación con las parcelas control de 1996. En 1997, sólo parcelas infestadas con 6 masas de huevecillos tuvieron un por ciento acumulado significativamente más alto de frutos dañados en comparación con las parcelas control. No hubo ninguna diferencia significativa entre los tratamientos en ambos años en el rendimiento de semilla de algodón.

The beet armyworm, *Spodoptera exigua* (Hübner), has been an occasional pest of cotton in the U.S. since the early 1900s (Sanderson 1905) causing damage primarily as a defoliator (Smith 1989, Leser et al. 1996). Injury to cotton associated with beet armyworm has traditionally been feeding on the foliage and flower buds, as well as etching on the bracts of fruiting forms (Smith 1989). These insects occasionally feed on squares and small bolls late in the growing season, but this injury typically has not resulted in economic yield losses, because fruiting forms that are produced late in the growing season generally do not significantly contribute to yield (Jenkins et al. 1990).

Beet armyworm population outbreaks occurred in the mid to late 1980's in Alabama (Smith 1989) and in 1993 in Mississippi (Layton 1994). In these areas, this pest uniformly infested fields, and larvae fed almost exclusively on squares, flowers, and young bolls during the fruiting stages of plant development rather than on foliage (Smith 1989, Layton 1994). During outbreaks in Alabama and Mississippi, many cotton producers sustained severe yield losses despite extensive control efforts, the cost of which exceeded $371 per hectare in some areas. Similar devastation by beet armyworm outbreaks has occurred in areas of Georgia (Douce & McPherson 1991, 1992) and Texas (Summy et al. 1996).

The economic impact of beet armyworm infestations include yield losses and costs associated with insecticide applications. Beet armyworms are tolerant to most registered classes of insecticides and control costs can become prohibitive when severe outbreaks occur (Layton 1994). The cost of insecticides targeted at beet armyworms exceeded $44 per application per hectare during the beet armyworm outbreaks in the Lower Rio Grande Valley of Texas in 1995 (Williams 1996).

Cotton production in Louisiana has not been threatened by severe beet armyworm outbreaks. Isolated economic infestations have been reported every two to three years since the mid 1980s (Burris et al. 1994), but cotton yield losses associated with beet armyworm injury have been less severe than in other states. However, during the past 4 years, this pest infested more than 500 thousand hectares of cotton in the state (Williams 1994, 1995, 1996, 1997).

Chlorpyrifos and thiodicarb are the only two insecticides currently recommended for beet armyworm control on cotton in Louisiana (Bagwell et al. 1997). Unsatisfactory efficacy of these insecticides against beet armyworm populations has been reported across most of the mid-south and southeastern U.S. (Elzen 1989, Burris et al. 1994, Layton 1994, Smith 1994, Graves et al. 1995, Mascarenhas et al. 1996, Sparks et al. 1996).

A Boll Weevil Eradication Program was implemented in Louisiana in August, 1997. The intensive insecticide regime associated with this program could release beet armyworms from their natural enemies (Evellens et al. 1973, Gaylor & Graham 1991, Ruberson et al. 1994), and cause widespread population outbreaks. The potential yield losses associated with beet armyworm damage to cotton have not been well studied. Therefore, the objective of this study was to determine the combined effects of late-season defoliation and fruit injury by beet armyworm on cotton yields.

Materials and Methods

Studies were conducted at the Northeast Research Station near St. Joseph, Louisiana during 1996 and 1997. Plots consisted of three adjacent rows (approximately 1 m centers) by 1.7 m in length covered by a translucent 32 mesh nylon cage (Synthetic Industries, Greenville, Georgia) measuring 1.7 × 3.4 × 1.7 m. Plots were planted to 'Stoneville 474' cotton, an early maturing variety, on 1 May in 1996 and on 4 June in 1997. In both years, plots were arranged in a randomized block design with 4 replications. Plots were treated as needed with insecticides to minimize defoliation and fruit damage by other insect pests. Insecticides that were selected for these applications were those with negligible activity against beet armyworms and short residual activity. Insecticide applications were initiated at first square and ended 7-10 d before plots were infested. Before covering the plots with cages, a combination of methyl parathion and acephate was applied to reduce populations of natural enemies within the caged area 24 h before artificial infestations.

Field-collected beet armyworm strains were used to infest plots. Beet armyworm larvae collected from cotton in Tift County, Georgia on 20 and 21 June were used in 1996, while larvae collected from cotton near St. Joseph, Louisiana on 7 and 8 August were used in 1997. Field-collected larvae were transported to the Department of Entomology at Louisiana State University Agricultural Center in Baton Rouge, LA and reared using an artificial wheat-germ and soybean protein diet (King & Hartley 1985). Egg masses of the F2 and F1 generation were used in field infestations during 1996 and 1997, respectively.

Cotton plots were infested when plants reached 5 nodes above white flower (NAWF) stage and had accumulated approximately 300 heat units (Oosterhuis et al. 1993). Heat unit accumulation was calculated according to Bagwell & Tugwell (1992). Infestations were made on 2 and 27 August in 1996 and 1997, respectively. The center row of each plot was artificially infested with 0, 1, 3, or 6 egg masses. Egg masses on wax paper oviposition sheets were attached with a paper clip to the lower surface of fully expanded leaves in the middle one-third of the cotton canopy. Larval numbers were thinned to approximately 60-80 insects per egg mass 2-3 d after larval hatching (DAH). Larval survival and development was monitored through the duration of the experiment.

All shed fruiting structures were removed from the soil surface within the cages immediately before infestation. Square and boll damage was estimated by collecting all shed fruiting structures two times per week and examining them for larval feeding. Shed fruiting forms were categorized into two groups based on feeding signs on the fruit. Fruiting forms that were shed but had no signs of fruit feeding were categorized

as undamaged, while those that were shed and had signs of fruit feeding were categorized as damaged. Fruiting forms in which the bracts were etched, but no feeding signs were evident on the petals or carpel walls were recorded as undamaged. Fruit damage was monitored until most larvae (>90%) had pupated in the soil (20-22 DAH).

Defoliation was estimated by measuring the photosynthetically active radiation (PAR) that penetrated through the cotton canopy. A 1-m light ceptometer (Decagon Devices, Inc. Pullman, Washington) probe equipped with 80 independent light sensors was used to measure PAR. All PAR sampling was conducted between the hours of 11:00 am and 1:30 pm to minimize the effects of sun position on PAR data. Six PAR samples were taken above the canopy by placing the ceptometer probe parallel to the top of the cages and perpendicular to the cotton rows. This measurement supplied the base level of PAR inside the cage. PAR samples below the canopy were taken by placing the probe perpendicular to the rows at the base of the cotton plants. Samples below the canopy were taken at 10 different locations within the cage. Sampling PAR above and below the canopy was conducted sequentially within a cage. Light penetration through the canopy was estimated by dividing the PAR below the canopy by the PAR above the canopy and multiplying that number by 100. Visual defoliation ratings after all larvae had pupated also were used to estimate foliage injury by beet armyworms. The percent of the leaf area consumed in infested plots were visually compared to that in the control plots. Cotton yields were determined by manually harvesting the plots and measuring seed cotton weights. Data were analyzed by ANOVA and means were separated according to Fisher's Protected LSD ($P = 0.05$) (SAS Institute 1988). Statistical comparisons ($\alpha = 0.05$) were done within sampling date and across infestation densities.

Results

Defoliation

Light penetration through the cotton canopy was significantly higher in plots infested with beet armyworm eggs masses compared with control plots on most sampling dates (Table 1). In 1996, all beet armyworm infested plots had significantly more (1.5 to 1.7-fold) light penetrating the canopy than the control plots at 9 DAH. At 13 DAH, all infested plots, except for those infested with 3 egg masses, had significantly more light penetration (1.3 to 1.5-fold) than the control plots. At 16 DAH, all infested plots, except for those infested with 1 egg mass, had significantly higher light penetration (1.2 to 1.4-fold) than the control plots. In 1997, only the plots infested with 3 egg masses had significantly more (1.5-fold) light penetrating the canopy than the control plots at 9 DAH. At the remaining sampling dates (12, 16, and 19 DAH), plots infested with 3 and 6 egg masses had significantly more light penetrating through the canopy than the control plots. At 12, 16, and 19 DAH, plots infested with 3 egg masses had 1.6, 1.8, and 1.9-fold more light penetration than the control plots, respectively. Similarly, plots infested with 6 egg masses had 1.6, 1.8, and 2.2-fold more light penetration than the control plots at 12, 16, and 19 DAH, respectively.

Visual defoliation ratings in 1996 showed that only the plots infested with 6 egg masses had significantly higher defoliation (14%) than the control plots (4%) (Table 1). In 1997, there were no significant differences in visual defoliation ratings between infested and control plots, despite a greater range in defoliation estimates.

Fruiting Form Damage 1996

Cumulative numbers of damaged fruiting forms in plots infested with 1, 3, or 6 egg masses was 2.4, 3.0, and 3.3-fold higher than that in the control plots (Fig. 1). Although

TABLE 1. LIGHT PENETRATION IN COTTON PLOTS INFESTED WITH 0, 1, 3, OR 6 BEET ARMYWORM EGG MASSES. PLOTS WERE INFESTED WHEN COTTON REACHED NAWF = 5 PLUS 300 HEAT UNITS.

Number of Egg Masses[1]	% Light Penetration[2] (1996)			% Light Penetration (1997)				Visual % Defoliation	
	9 DAH[3]	13 DAH	16 DAH	9 DAH	12 DAH	16 DAH	19 DAH	1996	1997
0	4.94 b	5.77 b	7.76 b	7.90 b	9.38 b	9.24 b	10.00 b	4.0 b	14.3 a
1	7.78 a	8.44 a	9.44 a	10.52 ab	11.52 ab	10.98 b	13.74 b	7.8 ab	32.5 a
3	7.47 a	7.66 ab	10.42 a	11.61 a	14.86 a	16.72 a	19.37 a	6.3 b	40.0 a
6	8.62 a	8.38 a	10.64 a	9.05 ab	14.86 a	16.67 a	22.37 a	13.8 a	56.7 a
F	3.4	2.8	3.0	2.9	4.7	12.1	14.9	4.2	0.9
df	(3,153)	(3,153)	(3,153)	(3,132)	(3,126)	(3,127)	(3,123)	(3,9)	(3,6)
P	0.02	0.04	0.03	0.04	<0.01	<0.01	<0.01	0.04	0.51

Means within a column not followed by a common letter differ significantly (Fisher's Protected LSD; $P = 0.05$).
[1]Larvae in each egg mass were thinned to 60-80 insects per egg mass.
[2]Light penetration measured in Photosynthetic Active Radiation (PAR) using light ceptometer. % Light penetration = (PAR bottom canopy/PAR top canopy) × 100.
[3]DAH = days after larval hatching.

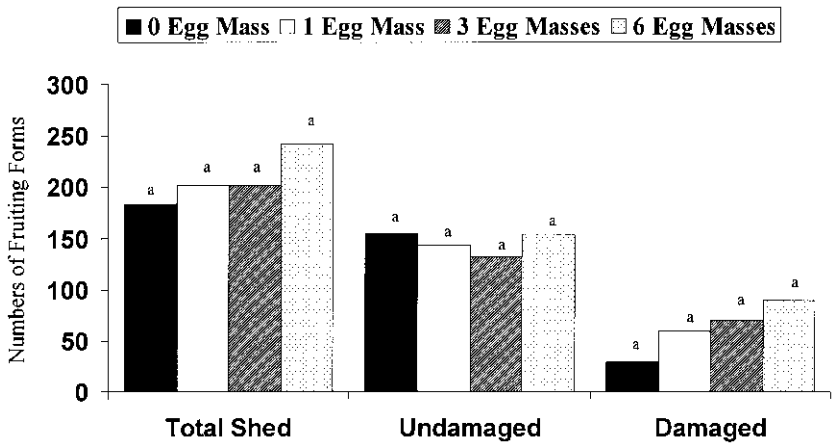

Fig. 1. Numbers of total shed, undamaged, and damaged fruiting forms in plots infested with 0, 1, 3, and 6 beet armyworm masses in 1996. Statistical comparisons within a category were made across egg mass densities. Different letters above bars indicate significant differences LSD (P = 0.05).

a trend for increased numbers of damaged fruiting forms with increases in egg mass density was observed, the cumulative numbers of damaged fruiting forms in infested plots were not significantly (α = 0.05) different from that in the control. In addition, there were no differences in the cumulative numbers of undamaged or total shed (undamaged + damaged) fruiting forms between infested and control plots (Fig. 1).

In all infested plots, a significantly higher percentage of the shed fruiting forms was damaged compared with the control plots at 6, 9, 13, and 16 DAH (Table 2). In addition, plots infested with 6 egg masses had a significantly higher percentage of shed fruiting forms that were damaged than the plots infested with 1 egg mass at 6 and 16 DAH. No differences were observed in the percentage of shed fruiting forms that were damaged between infested and control plots at 20 DAH. Similar results were obtained in the percentage of cumulative shed fruiting forms that were damaged (Table 2). All infested plots had a significantly higher percentage of the cumulative shed fruiting forms that were damaged than in the control plots.

Fruiting Form Damage 1997

The cumulative numbers of damaged fruiting forms in plots infested with 1, 3, or 6 egg masses were 2.1, 1.8, and 3.3-fold higher than that in the control plots (Fig. 2). Although a trend for increased numbers of cumulative damaged fruiting forms with increases in egg mass density was noted, no significant (α = 0.05) differences were observed. In addition, there were no significant differences in the cumulative numbers of undamaged or total shed fruiting forms between infested and control plots (Fig. 2).

The percentage of shed fruiting forms that were damaged tended to increase with increases in egg mass density. However, there were no significant differences among treatments on 3 of the 4 sampling dates. At 12 DAH, plots infested with 6 egg masses had a significantly higher percentage of the shed fruiting forms that were damaged

TABLE 2. PERCENT OF SHED FRUITING FORMS (SQUARES AND BOLLS) DAMAGED BY BEET ARMYWORM LARVAE IN PLOTS INFESTED WITH 0, 1, 3, AND 6 BEAT ARMYWORM EGG MASSES.

No. Egg Masses[1]	% Damaged Fruiting Forms (1996)[2]						% Damaged Fruiting Forms (1997)[2]				
	6 DAH[3]	9 DAH	13 DAH	16 DAH	20 DAH	Cumulative	9 DAH	12 DAH	16 DAH	20 DAH	Cumulative
0	10.9 c	18.4 b	14.7 b	16.5 c	9.9 a	16.3 c	2.4 a	3.2 b	6.4 a	1.0 a	3.2 b
1	18.7 b	36.5 a	35.0 a	29.5 b	15.6 a	31.5 b	10.0 a	3.4 b	33.3 a	25.0 a	6.0 b
3	21.7 ab	38.8 a	39.2 a	38.1 a	22.1 a	36.9 ab	16.4 a	6.7 b	31.3 a	33.3 a	22.2 b
6	25.3 a	36.3 a	42.9 a	40.4 a	19.5 a	38.3 a	26.4 a	36.5 a	25.0 a	41.7 a	54.0 a
F	17.3	12.4	15.6	19.1	1.5	23.3	2.2	.38	0.5	1.5	6.4
df	(3,9)	(3,9)	(3,9)	(3,9)	(3,9)	(3,9)	(3,9)	(3,9)	(3,9)	(3,9)	(3,9)
P	<0.01	<0.01	<0.01	<0.01	0.29	<0.01	0.16	0.05	0.74	0.28	0.01

Means within a column not followed by a common letter differ significantly according to Fisher's Protected LSD ($P = 0.05$).
[1]Numbers of larvae in each egg mass were thinned to 60-80 insects per egg mass.
[2]Percent damage fruiting forms = (No. damaged fruiting forms/No. total shed fruiting forms)* 100.
[3]DAH = days after larval hatching.

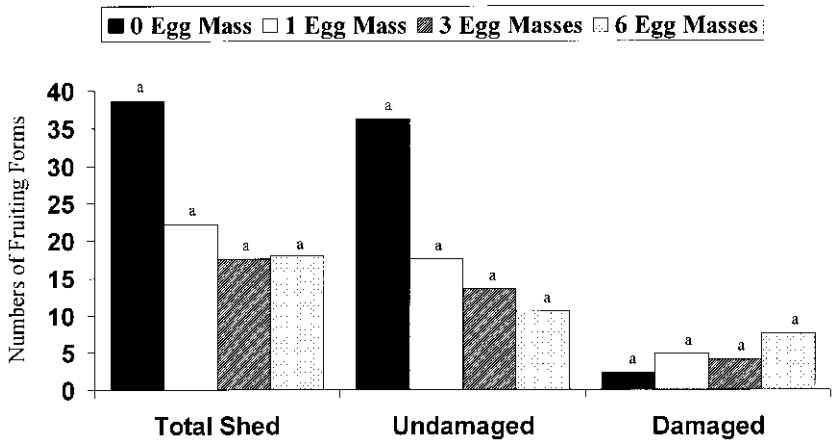

Fig. 2. Numbers of total shed, undamaged, and damaged from fruiting forms in plots infested with 0, 1, 3, and 6 beet armyworm egg masses in 1997. Statistical comparisons within a category were made across egg mass densities. Different letters above bars indicate significant differences LSD ($P = 0.05$).

than control plots, as well as plots infested with 1 or 3 egg masses (Table 2). Similar results were obtained in the percentage of cumulative numbers of shed fruiting forms that were damaged. Plots infested with 6 egg masses had a significantly higher percentage of the cumulative shed fruiting forms that were damaged than the control plots and those infested with 1 or 3 egg masses.

Yield

There were no significant differences ($\propto = 0.05$) in seed cotton yield between infested and non-infested control plots in 1996 or 1997 (Fig. 3).

Larval Development and Survival

Larval development and survival was normal in both years. Egg masses hatched 2-3 d after they were pinned to the leaves. Neonate larvae fed gregariously on the underside of leaves near the egg mass. Larvae began to disperse throughout the infested plant 3-4 d after they hatched, and 8 d after hatching, larvae were found throughout the cage environment. Larval survival to pupation was estimated at greater than 50%.

Discussion

Yield losses associated with beet armyworm damage may result from direct damage to fruiting forms, as well as indirect damage from larvae feeding on foliage. Foliage feeding can indirectly affect yield by reducing the leaf area that produces photosynthates required to mature bolls. In previous studies, Kerby et al. (1988) showed that cotton can withstand up to 57% defoliation (artificial removal of leaves) before first square without significant reduction in lint yield. Additionally, Russell et al. (1993) conducted simulated defoliation studies in which cotton was repeatedly de-

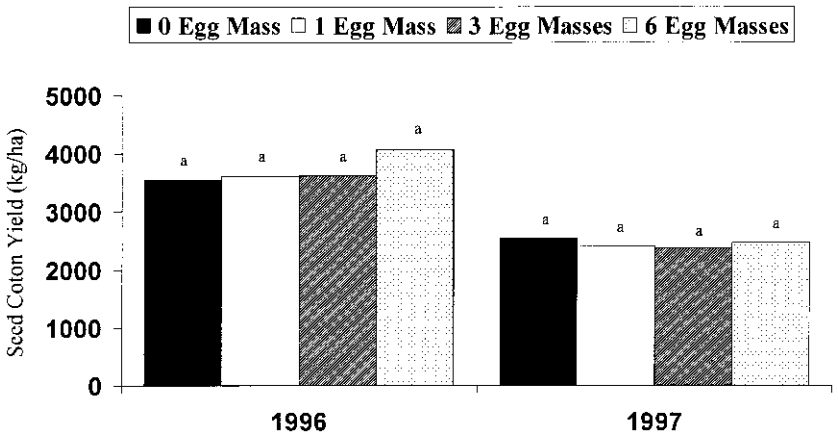

Fig. 3. Seed cotton yield in plots infested with 0, 3, and 6 beet armyworm egg masses in 1996 and 1997. Statistical comparisons were made across egg mass densities. Different letters above bars indicate significant differences LSD ($P = 0.05$).

foliated (20%) over a period of 7 consecutive weeks, from early squaring to mid-bloom, with no effect on yield. However, Russell et al. (1993) suggested that severe defoliation (>20%) during boll maturation could significantly impact yield by reducing the production of photosynthates in leaves necessary for maximum boll development.

The beet armyworm densities used in these studies ranged from 2.7 to 16.7-fold higher than the threshold of 6 hits (egg masses or clusters of small larvae) per 91.5 meter of row currently recommended in Louisiana (Bagwell et al. 1997). At the infestation densities used in these studies, a significant increase in the amount of light penetrating the canopy was generally observed in plots infested with 3 or 6 egg masses, which suggests a significant decrease in leaf area in those plots. Visual defoliation ratings in plots infested with 1, 3, or 6 egg masses were an average (1996 and 1997) of 2.1, 2.2, and 3.8-fold higher than the control plots, respectively. However, these levels of foliage loss at NAWF ≤ 5 plus 300 heat units were not sufficient to reduce yield in these plots compared with the control plots. These data are similar to research by Guitierrez et al. (1975), that showed cotton defoliation by beet armyworms and cabbage looper, *Trichoplusia ni* (Hübner), late in the growing season had little effect on yield. Similar results also were reported by Torrey et al. (1997) where removal of all foliage from the bottom 1/3 of the cotton canopy (33% defoliation) did not significantly reduce yields when plant development was at NAWF ≤ 5 plus 350 heat units. Results obtained in this study could have been caused by a compensatory effect (Oosterhuis et al. 1991), where plants were able to produce new leaf material at a rate in which the demands for photosynthates by the maturing bolls were met. Thus, no reduction in yield was observed. Alternatively, defoliation at this late stage of plant maturity (NAWF ≤ 5 plus 300 heat units) may not have affected yield because there was sufficient leaf area remaining to mature bolls. Cotton plants appear relatively unaffected by moderate (<40%) defoliation from early season to mid-flowering (Kerby et al. 1988, Russell et al. 1993). After plants have reached 5 NAWF and have accumulated heat units in excess of 300, late season defoliation low in the canopy also has little effect on yields (Torrey et al. 1997).

Although beet armyworms historically are recognized as defoliators, their direct feeding on fruiting forms often is of a much greater yield consequence (Smith 1989, Layton 1994). In 1996, a definite trend for increasing fruit damage occurred with increases in egg mass density. During the period that this study was conducted (one larval cycle or approximately 22 d), larvae in plots infested with 1, 3, or 6 egg masses damaged approximately 60, 70, and 90 fruiting forms, respectively (Fig. 1). However, these levels of fruit damage observed (1996) had no significant effect on yield. In fact, a trend for slight seed cotton yield increases with increased egg mass density was observed in 1996 (Table 1). A similar trend also was observed in the cumulative number of total shed fruiting forms (Fig. 1). In these studies, some level of fruit abscission may have occurred due to shading effects caused by the cage. Inside the cage, the light intensity was 60% of that recorded outside of the cage under direct sunlight. Guinn (1982) reported high rates (>90%) of fruit abscission when cotton plants were exposed to dim light (4 $\mu Em^{-2}s^{-1}$) for 3 d. Although abscission rates for young bolls (4-8 d old) was near 100%, abscission rates declined very rapidly for older bolls. Bolls that were 15 d past anthesis when exposed to dim light were virtually immune to abscission (Guinn 1982). In this study, a significant portion of undamaged young bolls did abscise in all treatments (Fig. 1). However, most of the older bolls were sufficiently matured (> 8 d old) and were not likely to abscise due to the shading caused by the cages. By having a slightly higher incidence of shed fruiting forms, plants in infested plots may have been able to concentrate their photosynthate resources on older fruiting forms, thus producing slightly bigger bolls than produced in the control plots. Small fruit abscission can be beneficial because it allows for the maturation of bigger bolls which the plant already has invested time and energy (Hake et al. 1989). The lack of differences in the cumulative numbers of total shed fruiting forms (Fig. 1), and the significantly higher percentage of shed fruiting forms damaged in infested plots (Table 2), indicates that the majority of the fruiting forms damaged by beet armyworm larvae were those that the plant would have naturally shed in the absence of insect damage.

The trends observed in fruit shedding and damage during 1996 were not repeated during 1997. A combination of late planting, poor early season growing conditions, and abnormally hot and dry late season growing conditions in 1997 likely impacted the outcome of this study. The overall yield potential of the plants was probably reduced due to stresses during the seedling and boll development stages. Differences in plant condition (fruit load and canopy mass) between 1996 and 1997 likely had some influence on the feeding behavior of beet armyworms.

The numbers of total shed fruiting forms was extremely different between the 1996 and 1997 studies. In the control plots, the number of total shed (undamaged + damaged) fruiting forms in 1996 was 4.8-fold higher than in 1997. Similarly, in plots infested with 1, 3, or 6 egg masses, the number of total shed fruiting forms in 1996 was 9.5, 11.5, and 13.5-fold higher than in 1997, respectively. Some of the fruiting forms in the 1997 test plots had suffered considerable damage from other insects and were aborted before cages were in place. This decreased the available numbers of fruiting forms susceptible to damage and shed from beet armyworm feeding. Most fruit shedding occurred at the first two sampling dates in all plots. After those fruiting forms were shed, remaining bolls may have been mature enough to avoid damage by beet armyworm larvae (Adamczyk et al. 1998). Nevertheless, there was a general trend for increased numbers of damaged fruiting forms with increases in egg mass density in 1997 (Fig. 2). However, no significant differences among treatments in seed cotton yield were observed in either year.

In summary, results indicate that neither defoliation nor fruit damage caused by late season beet armyworm infestation levels as high as 16.7 times the current threshold of 6 hits per 91.5 meters of row significantly affected cotton yields in these

studies. Further research is needed to determine the consequences of beet armyworm infestations that may occur earlier in the growing season, when cotton bolls may be more susceptible to damage by this pest. In addition, this research was conducted during only a single generation of the larval stage of this insect pest. Continuous damage caused by overlapping generations of beet armyworms can be considerably greater than that tested herein, thus potentially resulting in economic yield losses.

ENDNOTE

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TOXICITY OF SELECTED INSECTICIDES TO FALL ARMYWORMS (LEPIDOPTERA: NOCTUIDAE) IN LABORATORY BIOASSAY STUDIES

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ABSTRACT

Efficacy of conventional and experimental insecticides against the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), was evaluated in laboratory bioassays. In a laboratory diet bioassay, third instars of a laboratory-strain were more susceptible to novel insecticides, including chlorfenapyr, methoxyfenozide, spinosad, and tebufenozide, than to a recommended insecticide, thiodicarb. In other laboratory bioassays, fall armyworms were fed field grown cotton leaves, white flowers, or bolls treated with one of two recommended insecticides, L-cyhalothrin or thiodicarb, or one of four experimental insecticides, chlorfenapyr, emamectin benzoate, methoxyfenozide, or spinosad. First instar mortality was significantly greater on leaves treated with chlorfenapyr, L-cyhalothrin, or thiodicarb than for the untreated control at 24 h after infestation (HAI). First instar mortality was significantly greater on leaves treated with all insecticides, with the exception of methoxyfenozide, than for the untreated control at 48 HAI. Likewise, first instar mortality was significantly greater on white flowers treated with all insecticides, with the exception of methoxyfenozide, than for the untreated control at 24 HAI. First instar mortality on white flowers treated with all insecticides was significantly greater than the untreated control at 48 HAI. Fifth instar mortality on bolls was not significantly different among treatments at 1 day after infestation (DAI). At 3 and 5 DAI, fifth instar mortality was significantly greater on bolls treated with all insecticides, with the exception of methoxyfenozide and spinosad, than for the untreated control. At 7 DAI, fifth instar mortality was significantly greater on bolls treated with all insecticides, with the exception of spinosad, than for the untreated control. These data indicate that these recommended and experimental insecticides are effective in controlling early fall armyworm instars on cotton if larvae come in contact with these insecticides.

Key Words: *Spodoptera frugiperda*, efficacy, chemical control, insecticides

RESUMEN

La eficacia de insecticidas convencionales y experimentales contra el gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), se evaluó con bioensayos de laboratorio. En un experimento de dieta, se documentó que instares terceros de una colonia de laboratorio eran más susceptibles a los insecticidas nuevos, incluyendo a chlorfenapyr, methoxyfenozide, spinosad, y tebufenozide, que a un insecticida recomendado, thiodicarb. En otros experimentos de laboratorio, los gusanos cogolleros se alimentaron con hojas de algodón, flores blancas, o bellotas tratadas con uno de dos insecticidas recomendados, L-cyhalothrin o thiodicarb, o uno de cuatro insecticidas experimentales, chlorfenapyr, emamectin benzoate, methoxyfenozide, o spinosad. La mortalidad del primer instar fue significativamente mayor en hojas tratadas con chlorfenapyr, L-cyhalothrin, o thiodicarb que en hojas control no tratadas 24 h después de la infestacion (HAI, "hours after infestation"). La mortalidad del primer instar fue significativamente mayor a las 48 HAI en hojas tratadas con cualquiera de los insecticidas, con la excepción de methoxyfenozide, que con cualquier hoja control no tratada. Igualmente, la mortalidad del primer instar fue significativamente mayor a las 24 HAI en flores blancas tratadas con cualquiera de los insecticidas, con la excep-

ción de methoxyfenozide, que con el control. La mortalidad del primer instar a las 48 HAI en flores blancas tratadas con cualquiera de los insecticidas fue significativamente mayor que en los controles no tratados. La diferencia en la mortalidad del quinto instar en bellotas entre los tratamientos en el día 1 de la infestacion (DAI, "day of infestation") no fue significativa. En los DAI 3 y 5, la mortalidad del quinto instar fue significativamente mayor en bellotas tratadas con cualquiera de los insecticidas, con la excepción de methoxyfenozide y spinosad, que con el control no tratado. En el DAI 7, la mortalidad del quinto instar fue significativamente mayor en botones tratados con cualquiera de los insecticidas, con la excepción de spinosad, que en el control no tratado. Estos datos indican que los insecticidas recomendados y experimentales mencionados aquí son eficaces para el control de instares pequeños del gusano de *Spodoptera frugiperda* en algodón si las larvas contactan estos insecticidas.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a pest of many crops in the southern United States, including rice, *Oryza sativa* L.; field corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.; and cotton, *Gossypium hirsutum* L. (Young 1979). Historically, this insect is considered as a sporadic pest on cotton, but it has become an annual economic cotton pest in Georgia, Alabama, and Florida (Smith 1985). In 1977, this pest caused significant damage to cotton throughout the southeastern United States (Bass 1978), and in 1984, caused economic damage in the Winter Garden region of Texas (King et al. 1986). In 1985, it was the single most damaging cotton pest reported in Mississippi (King et al. 1986). Recently, local outbreaks of fall armyworms have been reported on transgenic *Bacillus thuringiensis* (Bt) cotton cultivars in Alabama and Georgia (Hood 1997, Smith 1997).

Fall armyworms on cotton are difficult to control with insecticides. Larvae are usually distributed low in the plant canopy (Ali et al. 1990), and inadequate insecticide deposition in the lower portions of the cotton plant seems to be one limiting factor in controlling this pest (Mink & Luttrell 1989). Insecticides that are used to control the tobacco budworm, *Heliothis virescens* (F.), and the cotton bollworm, *Helicoverpa zea* (Boddie), often are ineffective against fall armyworms (Smith 1985). Pyrethroids may have some effect on young fall armyworm larvae, but in general provide little overall control, while carbaryl and methyl parathion are completely ineffective for controlling this pest on cotton (Smith 1985). Although the CryIA (c) ∂-endotoxin is expressed throughout genetically engineered transgenic Bt cotton plants, this particular ∂-endotoxin should be classified as sublethal to fall armyworm larvae (Jenkins et al. 1992, Jenkins et al. 1997, Adamczyk et al. 1998). In addition, many studies have shown that fall armyworms are resistant to a number of compounds including carbaryl, methyl parathion, trichlorfon, and numerous pyrethroids (Young & McMillian 1979, Wood et al. 1981, McCord & Yu 1987, Yu 1991), which further complicates control of this pest on cotton. The purpose of these studies was to examine the toxicity of selected conventional and experimental insecticides to fall armyworm larvae in laboratory bioassays.

Materials and Methods

Diet Bioassay with Experimental Insecticides

A fall armyworm colony consisting of the corn-associated strain (Pashley 1986), which had been reared in the laboratory for at least 30 generations, (obtained from Dr. H.W. Fescemyer, Clemson University, Department of Entomology) was tested with a

recommended insecticide, thiodicarb (Larvin® 3.2F [flowable], Rhône-Poulenc Ag. Co., Research Triangle Park, North Carolina), as well as four novel compounds including chlorfenapyr (Pirate® 3SC [soluble concentrate], American Cyanamid, Princeton, New Jersey), methoxyfenozide (Intrepid® 80WP [wettable powder], Rohm & Haas Co., Philadelphia, Pennsylvania), spinosad (Tracer® 4SC, Dow AgroSciences, Indianapolis, Indiana), and tebufenozide (Confirm® 2F, Rohm & Haas Co., Philadelphia, Pennsylvania).

The surface-treated diet bioassay methods were similar to those described by Joyce et al. (1986), Chandler & Ruberson (1994), and Mascarenhas et al. (1996). Three ml of a soybean/protein meridic diet (King & Hartley 1985) were pipetted into 30 ml cups and allowed to cool at room temperature for approximately 1 h. For each insecticide tested, serial dilutions of formulated material (100 μl aliquots) were pipetted onto the diet surface, agitated to distribute evenly, and allowed to dry for approximately 30 min.

Third instar fall armyworms (30-45 mg) were placed into a series of cups that contained 4-5 different concentrations (ppm) of formulated insecticide, along with untreated controls to determine the LC_{50} for a given insecticide. Each cup contained one larva. The cups were sealed with corresponding lids and bioassays were conducted under constant light at 22 ± 1°C and 40 ± 5% RH. A minimum of 30 larvae per dose were tested for each insecticide, and mortality was assessed at 120 h after treatment (HAT). Larvae were considered dead if no movement was observed after prodding with blunt forceps for 10 s. Control mortality never exceeded 5%. LC_{50}'s were considered significantly different from one another if the 95% confidence limits did not overlap. Data were analyzed and LC_{50}'s generated with POLO-PC using probit analysis (LeOra Software 1987).

Toxicity of Cotton Plant Parts Treated with Insecticides to Fall Armyworm Larvae

Fall armyworm larvae were collected from field corn in May 1997 at the Macon Ridge Location of the Northeast Research Station (Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station) near Winnsboro, LA. F_1 generation larvae were used in all tests. Cotton plants (cv. DP 5690 ≈ 1.0 m tall) were sprayed with selected foliar insecticides using a high clearance sprayer, and compressed air system, calibrated to deliver 56.1 L total spray/ha through Teejet TX-8 hollow cone nozzles (2/row) at 3.3 kg/cm^2. Selected insecticides included methoxyfenozide, L-cyhalothrin (Karate® 1EC [emulsifiable concentrate], Zeneca Ag. Products, Wilmington, Delaware), thiodicarb, chlorfenapyr, emamectin benzoate (Proclaim® 5SG [soluble granule], Novartis, North Carolina), and spinosad. Treatments were arranged in a randomized complete block design (RCB) and replicated 4 times. Plots consisted of 4 rows (1.0 m centers) × 15.2 m. Cotton leaves, white flowers, and bolls, were removed from the lower 0.5 m of treated plants 2 HAT, and transported to the laboratory for each test.

The toxicity of insecticide-treated cotton leaves and white flowers to first instar (1-d-old) fall armyworms was evaluated. Five first position white flower subtending leaves, and entire white flowers, were removed from treated plants within the center 2 rows of each plot. Individual leaves were placed into 9.0 cm plastic Petri dishes, and entire white flowers were placed into individual 236.6 ml paper Solo® cups. A moistened filter paper was placed into each dish or cup to delay plant tissue desiccation. Fall armyworm larvae were reared on artificial diet for 24 hours to minimize disease effects, and healthy larvae transferred to treated leaves or white flowers. Five larvae were placed in each dish or cup (5 larvae/5 dishes or cups/plot, and replicated 4 times = 100 larvae/treatment) using a small camel-hair brush. The dishes or cups were sealed with corresponding lids. Both dishes and cups were maintained at 25º ± 1ºC and 40 ± 5% RH. Larval mortality was assessed at 24 and 48 h after infestation (HAI) and were considered dead if no movement was observed after being probed gently

with a camel-hair brush for 5 s. Mean mortality was calculated for each dish or cup, and these means were analyzed using ANOVA and treatments separated using the Waller-Duncan k-ratio t-test (PRM 1995).

The toxicity of insecticide-treated cotton bolls to fifth instar (200-350 mg) fall armyworms was evaluated using similar methods. Cotton bolls were age-classed using the methods described in Adamczyk et al. (1997a). White flowers from the lower 0.5 m from plants were tagged at anthesis and heat unit (HU) accumulation was recorded. Bolls had accumulated 187.0 HU at the time insecticides were applied. These bolls were removed from the plants and placed into individual 110.9 ml plastic cups. One fifth instar was placed in each cup (10 bolls/plot and replicated 4 times = 40 larvae/treatment), and the cups were sealed with corresponding lids. These containers were maintained in an environmental chamber at 26 ± 1ºC and a photoperiod of 14:10 (L:D) h. Larval mortality was assessed from 1-9 days after infestation (DAI) and were considered dead if no movement was observed after being probed with blunt forceps for 10 s. Results were analyzed using ANOVA and treatments separated using the Waller-Duncan k-ratio t-test (PRM 1995).

Results and Discussion

Diet Bioassay with Experimental Insecticides

The fall armyworm consists of two host-associated strains that widely differ in their susceptibility to insecticides (Adamczyk et al. 1997b). Therefore, it is essential that fall armyworm insecticide efficacy studies identify the host from which the test insects were collected. Our data contain baseline susceptibility information for corn-associated fall armyworms treated with four novel insecticides which can be used in the future for monitoring insecticide susceptibility. LC_{50} values ranged from 4.4 ppm for spinosad, to 492.9 ppm for thiodicarb (Table 1). The four novel insecticides (chlorfenapyr, methoxyfenozide, spinosad, and tebufenozide) were more toxic than the recommended insecticide, thiodicarb. The LC_{50} values for these new insecticides are similar to those reported by Mascarenhas et al. (1996) for the beet armyworm, *Spodoptera exigua* (Hübner), using these same methods.

Toxicity of Cotton Plant Parts Treated with Insecticides to Fall Armyworm Larvae

First instar mortality was significantly greater on cotton leaves treated with chlorfenapyr, L-cyhalothrin, or thiodicarb than for the untreated control at 24 HAI (Table 2). At 48 HAI, mortality for all treatments, with the exception of methoxyfenozide, was significantly greater than for the untreated control.

First instar mortality was significantly greater on white flowers for all treatments, with the exception of methoxyfenozide, than for the untreated control at 24 HAI (Table 2). At 48 HAI, mortality for all treatments was significantly greater than for the untreated control.

Fifth instar mortality on bolls was not significantly different among treatments at 1 DAI (Table 3). Larval mortality for all treatments, with the exception of methoxyfenozide and spinosad, was significantly greater than for the untreated control at 3 and 5 DAT. Fall armyworm larval mortality for all treatments, with the exception of spinosad, was significantly greater than for the untreated control at 7 DAT.

Most of the insecticides tested against fall armyworms were equally effective. While spinosad was very effective against first instars, the activity against fifth instars was numerically lower compared to methoxyfenozide, L-cyhalothrin, thiodicarb,

TABLE 1. SUSCEPTIBILITY OF THIRD INSTAR FALL ARMYWORMS FROM A LABORATORY STRAIN[1] AFTER FIVE DAYS OF EXPOSURE TO DIET TREATED WITH SELECTED INSECTICIDES.

			95% Confidence Limits			
Insecticide	No. Tested	LC_{50}[2] (ppm)	Low	High	Slopes (SE)	X^2
Chlorfenapyr	150	8.3	7.0	9.7	10.47 (1.75)	4.15
Methoxyfenozide	130	197.9	138.8	294.9	1.82 (0.42)	1.83
Spinosad	135	4.4	1.7	6.8	1.43 (0.42)	1.72
Tebufenozide	135	30.1	20.0	40.6	2.09 (0.44)	0.63
Thiodicarb	135	492.9	357.7	602.6	3.55 (0.74)	0.62

[1]Clemson University, Department of Entomology.
[2]LC_{50}'s significantly different if 95% confidence limits do not overlap.

chlorfenapyr, and emamectin benzoate. An insect growth regulator, methoxyfenozide, was very effective against both larval stages, but required considerably longer to maximize mortality compared to the other insecticides. In bioassays using field treated cotton parts, the pyrethroid, L-cyhalothrin, and carbamate, thiodicarb, were as effective as the newer compounds.

Our studies generally agree with the results of other fall armyworm research. Fall armyworms are susceptible to numerous insecticides if the larvae are exposed to the

TABLE 2. TOXICITY OF INSECTICIDE RESIDUES ON COTTON LEAVES AND WHITE FLOWERS[1] TO FIRST INSTAR FALL ARMYWORMS.

		% Mortality			
		Leaves		White Flowers	
Treatment	Rate (kg AI/ha)	24 HAI[2]	48 HAI	24 HAI	48 HAI
Chlorfenapyr	0.34	69.7 a	84.1 a	47.7 ab	87.3 ab
Emamectin benzoate	0.01	54.3 ab	81.5 a	67.0 a	92.0 a
L-cyhalothrin	0.04	54.7 a	77.6 a	74.4 a	91.4 ab
Methoxyfenozide	0.51	25.6 c	54.7 b	31.0 bc	77.3 b
Spinosad	0.10	52.6 ab	85.8 a	50.3 ab	94.8 a
Thiodicarb	0.84	58.6 a	87.8 a	54.8 ab	86.5 ab
Untreated		30.7 bc	43.7 b	11.8 c	44.6 c
F value		4.3	5.9	4.6	11.7
(*P* > *F*) ANOVA		<0.01	<0.01	<0.01	<0.01

Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$; Waller-Duncan k-ratio t-test).
[1]Leaves and white flowers removed 2 hours after insecticide treatment.
[2]Hours After Infestation.

TABLE 3. TOXICITY OF INSECTICIDE RESIDUES ON COTTON BOLLS[1] TO FIFTH INSTAR FALL ARMYWORMS.

Treatment	Rate (kg AI/ha)	% Mortality			
		1 DAI[2]	3 DAI	5 DAI	7 DAI
Chlorfenapyr	0.34	10.0 a	42.5 a	52.5 a	57.5 a
Em. benzoate	0.01	10.0 a	32.5 a	50.0 a	50.0 ab
L-cyhalothrin	0.04	20.0 a	32.5 a	40.0 ab	45.0 ab
Methoxyfenozide	0.51	5.0 a	10.0 b	35.0 abc	55.0 a
Spinosad	0.10	5.0 a	7.5 b	12.5 bc	22.5 bc
Thiodicarb	0.84	25.0 a	42.5 a	47.5 a	52.5 a
Untreated		0.0 a	2.5 b	7.5 c	15.0 c
F value		2.4	6.6	4.3	3.8
(*P* > *F*) ANOVA		0.07	<0.01	<0.01	<0.01

Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$; Waller-Duncan k-ratio t-test).
[1]Bolls removed 2 hours after insecticide treatment.
[2]Hours After Infestation.

insecticide (Mink & Luttrell 1989), but first instars are more susceptible to insecticides compared to later instars (Yu 1983). In addition, inadequate penetration of insecticide sprays to the lower portions of the cotton plant continues to be a limiting factor in controlling this pest (Mink & Luttrell 1989, Ali et al. 1990). Thus, it may be beneficial for a producer to manage excessive plant height with plant growth regulators (PGR s) in geographical areas where fall armyworms are an annual cotton pest.

ENDNOTE

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COMPARISONS OF LABORATORY AND FERAL STRAINS OF *SPODOPTERA FRUGIPERDA* AND *HELICOVERPA ZEA* (LEPIDOPTERA: NOCTUIDAE) IN LABORATORY AND FIELD BIOASSAYS

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ABSTRACT

The effects of resistant corn entries and resistant silk-diets on the growth and development of fall armyworm and corn earworm from a laboratory and a ¾ wild colony were compared in laboratory and field studies. For both species, there were significant interactions between insect strain and diet treatments. Compared to the laboratory strains, the ¾ wild strains produced lighter larvae and required longer developmental times when reared on diets with and without resistant silks. Larval growth of both insect strains was significantly retarded by the addition of resistant silks to the diets. In field studies, the ¾ wild strains generally performed better than the laboratory strains. For both insect species, interactions between insect strains and corn lines were observed. Strain differences for all measured parameters were greater for the corn earworm than for the fall armyworm. Results from these studies indicate that research on plant resistance for the fall armyworm and the corn earworm would better predict the relative levels of resistance among different corn lines and among different silk diets if ¾ wild colonies were established annually, and if insects from these ¾ wild colonies were used in conducting laboratory and field bioassays.

Key Words: Plant Resistance; maize; corn silks; meridic diets

RESUMEN

Los efectos de la introducción de líneas resistentes de maíz y de dietas con estigmas resistentes en el crecimiento y desarrollo del gusano cogollero del maíz y del gusano del elote del maíz de una colonia de laboratorio y de una colonia ¾ salvaje fueron comparadas en estudios de laboratorio y de campo. Para ambas especies se notaron interacciones significativas entre el tipo de insecto y los tratamientos de dieta. En comparación con las colonias de laboratorio, las colonias ¾ salvajes produjeron larvas más ligeras y que requirieron períodos de desarrollo más largos cuando se criaron en dietas con o sin estigmas resistentes. El crecimiento larval de ambos tipos de insecto fue significativamente retardado por la adición de estigmas resistentes a las dietas. En estudios de campo, las colonias ¾ salvajes resultaron mejores que las colonias de laboratorio. En ambas especies se observaron interacciones entre los tipos de insecto y las líneas de maíz. En todos los parámetros medidos las diferencias entre las colonias fueron más grandes para el gusano del elote que para el gusano cogollero. Los resultados de estos estudios indican que investigaciones sobre resistencia del maíz contra el gusano cogollero y el gusano del elote podrían predecir mejor los niveles relativos de resistencia entre líneas diferentes de maíz y entre diferentes dietas de estigmas si se establecieran anualmente colonias ¾ salvajes y si insectos de estas colonias ¾ salvajes se utilizaran para conducir bioensayos de laboratorio y de campo.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and the corn earworm, *Helicoverpa zea* (Boddie), are two of the most destructive pests of corn, *Zea mays* L., in the United States. The use of corn varieties resistant to these insect pests is an ideal method to reduce losses caused by insect feeding and to reduce the population density of insect pests developing on corn. Resistant corn varieties can be used as the primary method of insect control or as a component of an integrated pest management scheme (Wiseman et al. 1983). Corn germplasm resistant to the fall armyworm (Wiseman et al. 1976, Williams & Davis 1997a, Wiseman et al. 1981) and to the corn earworm (Straub & Fairchild 1970, Wiseman & Davis 1990) have been discovered.

Rearing fall armyworm and corn earworm in laboratory colonies has been an important part of research programs developing corn varieties resistant to these insect pests (Davis & Guthrie 1992). Laboratory reared insects are used to artificially infest corn plants in the field, and fresh leaves and silk diets and reconstituted leaf and silk diets in the laboratory (Davis et al. 1989, Williams & Davis 1997b, Wiseman et al. 1981, Wiseman et al. 1983, Wiseman & Wilson 1987). Although natural populations or field collections of fall armyworm and corn earworm can be useful to researchers in plant resistance, laboratory colonies of these pests provide a reliable source of insects for these studies and thereby allow for an expanded research program. Because laboratory colonies of fall armyworm and corn earworm are relied upon by many researchers to conduct plant resistance studies, it is important that the insects in the laboratory colonies are physiologically and behaviorally equivalent to their wild counterparts (Davis & Guthrie 1992). The infusion of new genes from wild insects into laboratory colonies can improve the field performance of laboratory-reared insects (Young et al. 1975). As a precautionary measure, some researchers start new laboratory colonies or infuse new genes into their laboratory colony each year (Davis & Guthrie 1992). In this study, our objectives were to compare the performance of a laboratory colony and a ¾ wild colony of the corn earworm and fall armyworm when reared on silk-diets or on corn plants in the field with varying levels of resistance.

Materials and Methods

Laboratory corn earworm and fall armyworm larvae were obtained from cultures maintained on a corn-soy-milk solids and pinto bean diets, respectively, (Perkins 1979; Burton & Perkins 1989) at the Insect Biology and Population Management Research Laboratory, Tifton, GA. The laboratory corn earworm culture is sustained in a heterozygous state by maintaining a series of carefully controlled crosses (Young et al. 1976). A ¾ wild strain was developed for both the corn earworm and fall armyworm by crossing wild males with laboratory females. Female progeny (½ wild) from these crosses were mated with wild males (Young et al. 1975). Wild corn earworm males were collected in light traps during early and late October, 1996. Wild fall armyworm males were collected as larvae from whorl corn during late August and late September, 1996.

Laboratory studies were conducted on the ¾ wild, corn earworm strain during the 2nd and 3rd generation. Field studies were conducted on the ¾ wild, corn earworm strain during the 5th and 6th generation. Laboratory studies were conducted on the ¾ wild, fall armyworm strain during the 3rd and 4th generation, and field studies were conducted during the 7th and 8th generations.

Two laboratory experiments, one for the corn earworm and the other for the fall armyworm, were conducted as a split plot design with 30 replications and 1 cup per replication. Whole plots were the laboratory strain insects and the ¾ wild strain insects, and subplots were diet treatments. Diets were made using 50 and 25 mg 'Zapalote

Chico' (resistant) and 50 mg 'Stowell's Evergreen' sweet corn oven dried silks mixed (per 1 ml diluted diet) in pinto bean diet diluted at a rate of 3 ml of bean diet/2 ml of water. Controlled diets for each experiment were regular pinto bean diet (Burton & Perkins 1989) and a celufil check at the rate of 50 mg celufil/ml of dilute diet. The diet mixtures were dispensed into 30 ml plastic diet cups of ≈10 ml per cup. The diets were allowed to cool for ≈2 h, after which 1 neonate was introduced into each cup and the cup was capped. Weight of larvae (8 d for the corn earworm and 9 d for the fall armyworm), days to development to pupation and weight of pupae were recorded. Both experiments were held in a controlled environment room maintained at 28 ± 2°C and 75 ± 2% RH with a photoperiod of 14:10 (L:D).

Two field experiments with two planting dates each were conducted in 1997 for the corn earworm and fall armyworm. Four dent corn entries (resistant 'MpSWCB-4' and 'GT-FAWCC(C5)' or susceptible 'Cacahuacintle X's' and 'Pioneer 3369A') were selected for comparison against the fall armyworm. Zapalote Chico and 'Zimmerman Z-63W' (resistant) and Stowell's Evergreen and Pioneer 3369A (susceptible) were selected for comparison against the corn earworm.

The fall armyworm tests were seeded on 13 May, 1997 and 29 May, 1997 at Tifton, GA. The corn earworm tests were seeded on 2 April, 1997 and 22 April, 1997 at Tifton, GA. Test plots consisted of single rows 6.1 m long and 0.9 m apart. Plants were thinned to ca. 30 cm apart. Recommended agronomic practices were followed for both tests and planting dates.

A split plot design with 6 replications was used with whole plots being a check plot with no infestation, infested with the ¾ wild strain and infested with the laboratory culture of the fall armyworm or corn earworm, respectively. Subplots were corn entries.

Whorl stage plants (10 leaves) were infested with a total of 30 fall armyworm neonates (2 applications of 15/plant on the same day) using the 'Bazooka' method (Wiseman 1989). Counts of larvae and weight of biomass were made per 5 plants at 7 d after infestation (DAI) and rated at 7 and 14 DAI using a visual rating scale of 0-9 (Davis et al. 1992), where 0 = no damage and 9 = whorl destroyed.

Corn earworm larvae were infested on two-day-old silks at the rate of 5 larvae/silk mass. Counts of larvae and weight of their biomass per 5 ears were made at 7 DAI and injury ratings were made at 18 DAI (Wiseman 1989).

Data from laboratory tests and field tests were analyzed by PROC GLM (SAS Institute 1989). When significant differences were indicated, means were separated by least significant differences (LSD) at $P = 0.05$ (SAS Institute 1989).

Results and Discussion

Laboratory Experiments

Studies with the corn earworm revealed a significant interaction between insect strain and diet treatments for the 8-d larval weights (Table 1). Larvae from the laboratory colony performed significantly better on the bean diet than on the celufil and susceptible diets. Larvae from the ¾ wild colony performed significantly better on the celufil diet than on the bean and susceptible diets. Larval growth of both insect strains was significantly retarded by the addition of resistant silks to the diets. In general, the 8-d larval overall diet weights of the ¾ wild strain were about half the 8-d larval weights of the laboratory strain. The mean 8-d larval weight across diet treatments was 169.4 mg for the laboratory strain and 80.3 mg for the ¾ wild strain. There was a significant interaction between insect strain and diet treatments for the devel-

TABLE 1. EFFECT OF DIET TREATMENTS AND CORN EARWORM STRAIN ON WEIGHT (MG) OF 8-D-OLD LARVAE, DEVELOPMENTAL TIME OF LARVAE (DAYS TO PUPATION), AND PUPAL WEIGHT (MG).

Insect Strain	Diet Treatment[1]					Mean
	BNCK	CLCK	SEG25	ZC25	ZC50	
	Weight (mg) of 8-d-old larvae					
Laboratory	278.9 Aa	221.4 Ab	202.9 Ab	102.3 Ac	32.8 Ad	169.4
¾ Wild	103.6 Bb	139.9 Ba	106.5 Bb	36.1 Bc	7.6 Ad	80.3
Mean	201.4	180.6	159.2	70.5	20.9	
	Developmental time of larvae (days to pupation)					
Laboratory	13.1 Ad	14.3 Acd	15.4 Ac	17.7 Ab	26.9 Aa	17.0
¾ Wild	18.3 Bc	18.8 Bc	18.9 Bc	21.2 Bb	37.3 Ba	21.6
Mean	15.1	16.4	16.9	19.3	30.7	
	Pupal weight (mg)					
Laboratory	554.6 Aa	531.3 Aa	528.9 Aa	467.8 Ab	287.3 Ac	484.9
¾ Wild	398.5 Bb	414.9 Bab	432.2 Bab	437.3 Aa	321.7 Ac	409.0
Mean	495.6	478.6	488.3	453.9	299.8	

[1]BNCK = Bean check diet; CLCK = Celufil check; SEG25 = 'Stowell's Evergreen' 25 mg silks; ZC25 = 'Zapalote Chico' 25 mg silks; ZC50 = 'Zapalote Chico' 50 mg silks. Horizontal means followed by the same lowercase letter are not significantly different, and column means for each parameter followed by the same uppercase letter are not significantly different (Pœ0.05) as separated by LSD (SAS Institute 1989).

opmental time of corn earworm to pupation (Table 1). The laboratory strain required significantly more time to develop on diet containing susceptible silks than on the bean diet; however, there was no difference in the developmental time for the ¾ wild strain on these two diets. The number of days to pupation for both insect strains was significantly increased by the addition of resistant silks to the diets. The ¾ wild strain required a greater number of days to pupate on each diet treatment than did the laboratory strain. There also was a significant interaction between insect strain and diet treatments for the weight of corn earworm pupae (Table 1). Addition of resistant silks to the diet significantly reduced the weight of pupae for the laboratory strain. However, ¾ wild strain pupae that developed on the diet with the lower concentration of resistant silks were significantly heavier than pupae that developed on the bean diet. Except for the diet with the higher concentration of resistant silks, each diet treatment yielded heavier laboratory strain pupae than ¾ wild strain pupae.

Studies with the fall armyworm also showed a significant interaction between insect strain and diet treatments for the 9-d larval weights (Table 2). Larvae from both fall armyworm strains performed significantly better on the bean diet than on the celufil and susceptible diets. Larval growth of both insect strains was significantly retarded by the addition of resistant silks to the diets. Larvae from the ¾ wild strain weighed about 84% the weight of larvae from the laboratory strain when reared on the bean diet, and weighed about 50% the weight of larvae from the laboratory strain when reared on the resistant silk-diets. There was a significant interaction between insect strain and diet treatments for the developmental time of fall armyworm to pupation (Table 2). The number of days to pupation for both insect strains was signifi-

TABLE 2. EFFECT OF DIET TREATMENTS AND FALL ARMYWORM STRAIN ON WEIGHT OF 9-D-OLD LARVAE, DEVELOPMENTAL TIME (DAYS TO PUPATION), AND PUPAL WEIGHT (MG).

Insect Strain	Diet Treatments[1]					Mean
	BNCK	CLCK	SEG25	ZC25	ZC50	
	Weight (mg) of 9-d-old larvae					
Laboratory	212.0 Aa	187.1 Ab	155.5 Ac	35.1 Ad	6.1 Ae	119.2
¾ Wild	177.1 Ba	148.4 Bb	92.1 Bc	17.4 Ad	3.2 Ad	87.6
Mean	194.5	167.8 a	123.8	26.2	4.6	
	Developmental time of larvae (days to pupation)					
Laboratory	14.4 Aa	15.5 Aab	16.1 Ab	20.4 Ac	27.2 Ad	18.7
¾ Wild	15.9 Ba	17.1 Bab	17.9 Bb	22.2 Bc	33.7 Bd	21.4
Mean	15.2	16.2	17.0	21.3	30.4	
	Pupal weight (mg)					
Laboratory	305.4 A	273.2 A	272.6 A	243.4 A	159.9 A	159.7 a
¾ Wild	297.9 A	281.7 A	297.4 B	247.1 A	176.7 A	274.5 b
Mean	301.3 a	277.5 b	285.7 b	245.3 c	165.8 d	

[1]BNCK = Bean check diet; CLCK = Celufil check; SEG25 = 'Stowell's Evergreen' 25 mg silks; ZC25 = 'Zapalote Chico' 25 mg silks; ZC50 = 'Zapalote Chico' 50 mg silks. Horizontal means followed by the same lowercase letter are not significantly different, and column means for each parameter followed by the same uppercase letter are not significantly different ($P \leq 0.05$) as separated by LSD (SAS Institute 1989).

cantly increased by the addition of resistant silks to the diets. Developmental time on bean diet for ¾ wild larvae was about 1.5 d longer than the developmental time for laboratory larvae. When larvae were reared on the more resistant silk-diet, the developmental time for ¾ wild larvae was about 6.5 d longer than the developmental time for laboratory larvae. Pupae from the ¾ wild strain were significantly heavier than pupae from the laboratory strain (Table 2). Larvae that developed on bean diet produced significantly heavier pupae than did larvae that developed on the celufil and susceptible silk diets. Resistant silk diets produced significantly lighter pupae than the other diet treatments.

Field Experiments

The number of corn earworm larvae collected from 5 corn ears 7 d after infestation was significantly influenced by insect strain and corn line (Table 3). There was a significant interaction between corn line and insect strain for the first planting date but not for the second planting date. For each planting date, more larvae were found in the ¾ wild strain treatment than in the laboratory strain treatment. More larvae were produced on Stowell's Evergreen than the other corn lines for the first planting date, and more larvae were produced on P3369A than the other corn lines for the second planting date. The weight of larvae per 5 corn ears was similar to the number of larvae per 5 corn ears. Again, there was a significant interaction between corn line and insect strain for the first planting date but not for the second planting date (Table 4). Also,

TABLE 3. EFFECT OF CORN LINES AND CORN EARWORM STRAIN ON LARVAL SURVIVAL AT 7 DAYS AFTER INFESTATION (NO. OF LARVAE/5 EARS).

	Corn Line[1]				
Insect Strain	SEG	ZC	Z63W	P3369A	Mean
	First Planting Date (2 April)				
Check	3.3 Aa	1.5 Aa	1.3 Aa	1.8 Aa	2.0
Laboratory	8.2 Ba	2.2 Ab	2.8 ABb	2.8 Ab	4.0
¾ Wild	13.3 Ca	4.5 Ab	5.5 Bb	3.8 Ab	6.8
Mean	8.3	2.7	3.2	2.8	
	Second Planting Date (22 April)				
Check	3.2 Ab	0.2 Ac	7.0 Aa	9.8 Aa	5.0 A
Laboratory	7.2 Ba	1.8 Ab	6.3 Aa	8.3 Aa	5.9 AB
¾ Wild	11.3 Ca	2.8 Ab	7.5 Ab	10.5 Aa	8.0 B
Mean	7.2 b	1.6 c	6.9 b	9.6 a	

[1]SEG = 'Stowell's Evergreen'; ZC = 'Zapalote Chico'; Z63W = 'Zimmerman Z-63W'; P3369A = 'Pioneer 3369A'. Horizontal means followed by the same lowercase letter are not significantly different, and column means for each planting date followed by the same uppercase letter are not significantly different ($P \leq 0.05$) as separated by LSD (SAS Institute 1989).

for each planting date, the weight of larvae per 5 corn ears was greater for the ¾ wild strain than for the laboratory strain, however, the difference was not significant. For each planting date, the weight of larvae per 5 corn ears was significantly greater for

TABLE 4. EFFECT OF CORN LINES AND CORN EARWORM STRAIN ON WEIGHT OF LARVAE 7 DAYS AFTER INFESTATION [WEIGHT (MG) OF LARVAE/5 EARS].

	Corn Line[1]				
Insect Strain	SEG	ZC	Z63W	P3369A	Mean
	First Planting Date (2 April)				
Check	115.7 Aa	56.3 Aa	2.7 Aa	15.3 Aa	47.5
Laboratory	274.8 Ba	30.3 Ab	86.8 Ab	32.3 Ab	106.1
¾ Wild	349.5 Ba	126.5 Abc	24.0 Abc	9.7 Ac	127.4
Mean	246.7	71.1	37.8	19.1	
	Second Planting Date (22 April)				
Check	222.7 Aa	0.5 Ab	99.7 Aab	152.5 Aab	118.8 A
Laboratory	244.3 Aa	21.2 Ab	179.7 Aab	135.5 Aab	145.2 A
¾ Wild	321.5 Aa	192.7 Aab	81.8 Ab	100.0 Ab	174.0 A
Mean	262.8 a	71.4 b	120.4 b	129.3 b	

[1]SEG = 'Stowell's Evergreen'; ZC = 'Zapalote Chico'; Z63W = 'Zimmerman Z-63W'; P3369A = 'Pioneer 3369A'. Horizontal means followed by the same lowercase letter are not significantly different, and column means for each planting date followed by the same uppercase letter are not significantly different ($P \leq 0.05$) as separated by LSD (SAS Institute 1989).

Stowell's Evergreen than for the other corn lines. Measurements of the depth of ear penetration 18 d after infestation for the first planting date revealed that larvae from the ¾ wild strain penetrated significantly deeper into the ear than did the larvae from the laboratory strain or larvae from natural infestation (Table 5). Depth of ear penetration was significantly greater for Stowell's Evergreen than for the other corn lines. Results of ear penetration for the second planting date was similar except that there was a significant interaction between corn line and insect strain.

Results from the field study with fall armyworm showed significant interactions between corn lines and insect strain for each of the measured parameters for each planting date. For the first planting date the mean number of ¾ wild larvae per 5 plants was about twice the number of laboratory larvae per 5 plants (Table 6). The mean number of larvae per plant for the second planting date was similar for the ¾ wild and laboratory strains (18.6 and 21.9, respectively). No larvae were found in the check plots. More larvae were found in the susceptible corn plots than in the resistant corn plots for both planting dates. The weight of larvae per 5 plants for each corn line was greater for the laboratory strain than for the ¾ wild strain for the second planting date (Table 7). Also, the weight of larvae per 5 plants was greater for susceptible corn lines than for resistant corn lines. Weight of larvae per 5 plants was not recorded for the first planting date. The 7-d visual damage rating for the ¾ wild strain was greater than the 7-d visual damage rating for the laboratory strain for the first planting date (Table 8). For the second planting date the 7-d visual rating was similar for the ¾ wild and laboratory strains (2.5 and 2.7, respectively). No damage was found in the check plots. Damage ratings were higher in the susceptible corn plots than in the resistant corn plots for both planting dates. The visual damage ratings after 14 d were higher in each case than the damage ratings taken after 7 d (Table 9). Otherwise, the 14-d damage ratings were similar to the 7-d damage ratings when comparing between insect strains and among corn lines.

TABLE 5. EFFECT OF CORN LINES AND CORN EARWORM STRAIN ON DEPTH (CM) OF LARVAL PENETRATION INTO THE EAR 18 DAYS AFTER INFESTATION.

	Corn Line[1]				
Insect Strain	SEG	ZC	Z63W	P3369A	Mean
	First Planting Date (2 April)				
Check	3.8 Aa	2.6 Ab	1.6 Ac	2.5 Abc	2.5 A
Laboratory	3.6 Aa	2.5 Ab	1.9 Ab	2.8 Aab	2.7 A
¾ Wild	5.1 Ba	3.1 Ab	2.7 Ab	2.8 Ab	3.4 B
Mean	4.1 a	2.7 b	2.1 c	2.5 bc	
	Second Planting Date (22 April)				
Check	5.5 Aab	2.6 Ac	4.7 Ab	6.1 Aa	4.7
Laboratory	7.6 Ba	3.2 ABc	4.8 Ab	5.9 Ab	5.4
¾ Wild	8.1 Ba	4.4 Bb	5.1 Ab	5.8 Ab	5.9
Mean	7.1	3.4	4.9	5.9	

[1]SEG = 'Stowell's Evergreen'; ZC = 'Zapalote Chico'; Z63W = 'Zimmerman Z-63W'; P3369A = 'Pioneer 3369A'. Horizontal means followed by the same lowercase letter are not significantly different, and column means for each planting date followed by the same uppercase letter are not significantly different ($P \leq 0.05$) as separated by LSD (SAS Institute 1989).

TABLE 6. EFFECT OF CORN LINES AND FALL ARMYWORM STRAIN ON LARVAL SURVIVAL AT 7 DAYS AFTER INFESTATION (NO. OF LARVAE/5 PLANTS).

	Corn Line[1]				
Insect Strain	CACAH	GT-FAWCC (C5)	MpSWCB-4	P3369A	Mean
		First Planting Date (13 May)			
Check	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0
Laboratory	39.3 Ba	12.8 Bb	7.3 A	48.2 Ba	26.9
¾ Wild	78.7 Ca	40.8 Cc	27.8 Bd	58.8 Bb	51.7
Mean	39.3	16.5	11.7	34.3	
		Second Planting Date (29 May)			
Check	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0
Laboratory	34.8 Ba	14.0 Bc	13.2 Bc	25.7 Bb	21.9
¾ Wild	29.5 Ba	10.7 Bb	10.5 Bb	23.8 Ba	18.6
Mean	21.4	8.2	7.9	16.5	

[1]CACAH = 'Cacahuacintle'; GT-FAWCC(C5) = Registered maize population; MpSWCB-4 = Registered maize population; P3369A = 'Pioneer 3369A'. Horizontal means followed by the same lowercase letter are not significantly different, and column means for each planting date followed by the same uppercase letter are not significantly different ($P \leq 0.05$) as separated by LSD (SAS Institute 1989).

The use of insects reared on artificial diet in the laboratory is critical to the development of an efficient and complete plant resistance research program (Davis & Guthrie 1992). However, once an insect colony has been established in the laboratory it becomes incumbent upon the researcher to monitor and maintain the quality of the colony. Quality control should be a system that incorporates feedback information from colonization effects, colony management, and field evaluation (Dickerson & Lep-

TABLE 7. EFFECT OF CORN LINES AND FALL ARMYWORM STRAIN ON LARVAL SURVIVAL AT 7 DAYS AFTER INFESTATION [WEIGHT(MG) OF LARVAE/5 PLANTS].

	Corn Line[1]				
Insect Strain	CACAH	GT-FAWCC (C5)	MpSWCB-4	P3369A	Mean
		Second Planting Date (29 May)			
Check	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0
Laboratory	124.3 Ca	31.2 Bb	39.0 Bb	101.3 Ca	75.5
¾ Wild	95.3 Ba	28.0 Bb	23.5 ABb	71.2 Ba	54.4
Mean	73.2	19.7	19.8	57.5	

[1]CACAH = 'Cacahuacintle'; GT-FAWCC(C5) = Registered maize population; MpSWCB-4 = Registered maize population; P3369A = Pioneer 3369A. Horizontal means followed by the same lowercase letter are not significantly different, and column means followed by the same uppercase letter are not significantly different ($P \leq 0.05$) as separated by LSD (SAS Institute 1989).

TABLE 8. EFFECT OF CORN LINES AND FALL ARMYWORM STRAIN ON CORN DAMAGE (7-D VISUAL RATINGS).

	Corn Line[1]				
Insect Strain	CACAH	GT-FAWCC (C5)	MpSWCB-4	P3369A	Mean
	First Planting Date (13 May)				
Check	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0
Laboratory	4.3 Ba	3.2 Bb	2.3 Bc	5.0 Ba	3.7
¾ Wild	5.8 Ca	4.6 Cb	3.8 Cb	6.2 Ca	5.1
Mean	3.4	2.5	2.1	3.6	
	Second Planting Date (29 May)				
Check	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0
Laboratory	3.2 Bab	2.3 Bbc	1.5 Bc	3.8 Ca	2.7
¾ Wild	3.3 Ba	2.0 Bb	1.7 Bb	3.0 Ba	2.5
Mean	2.2	1.4	1.1	2.3	

[1]CACAH = 'Cacahuacintle'; GT-FAWCC(C5) = Registered maize population; MpSWCB-4 = Registered maize population; P3369A = 'Pioneer 3369A'. Horizontal means followed by the same lowercase letter are not significantly different, and column means for each planting date followed by the same uppercase letter are not significantly different ($P \leq 0.05$) as separated by LSD (SAS Institute 1989).

TABLE 9. EFFECT OF CORN LINES AND FALL ARMYWORM STRAIN ON CORN DAMAGE (14-D VISUAL RATINGS).

	Corn Line[1]				
Insect Strain	CACAH	GT-FAWCC (C5)	MpSWCB-4	P3369A	Mean
	First Planting Date (13 May)				
Check	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0
Laboratory	8.2 Ba	4.8 Bb	4.5 Bb	8.8 Ba	6.6
¾ Wild	8.5 Ba	6.0 Cb	5.7 Cb	8.6 Ba	7.2
Mean	5.6	3.5	3.4	5.6	
	Second Planting Date (29 May)				
Check	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0
Laboratory	4.8 Ba	2.8 Bb	3.2 Bb	5.8 Ca	4.2
¾ Wild	4.7 Ba	2.5 Bb	3.0 Bb	4.5 Ba	3.7
Mean	3.2	1.8	2.1	3.4	

[1]CACAH = 'Cacahuacintle'; GT-FAWCC(C5) = Registered maize population; MpSWCB-4 = Registered maize population; P3369A = 'Pioneer 3369A'. Horizontal means followed by the same lowercase letter are not significantly different, and column means for each planting date followed by the same uppercase letter are not significantly different ($P \leq 0.05$) as separated by LSD (SAS Institute 1989).

pla 1992). When the insect colony is no longer comparable to the wild population, researchers should consider strengthening the colony by the infusion of wild genes or replacing the colony with wild insects (Davis & Guthrie 1992). In deciding whether or not to strengthen an established laboratory colony, researchers should consider the goal of the proposed studies and the type of insect required to meet those research goals. Often, however, adequate research has not been conducted to guide the researcher in making these decisions.

Our studies indicate that research on plant resistance for the fall armyworm and the corn earworm would better predict the relative levels of resistance among different corn lines and among different silk diets if ¾ wild colonies were established annually, and if insects from these ¾ wild colonies were used in conducting laboratory and field bioassays. Caution should be exercised before discarding an established laboratory colony, however. Compared to a laboratory colony, a newly established ¾ wild strain may be less robust in laboratory culture and, therefore, less reliable in producing an adequate supply of insects for research purposes. Also, insect pathogens could be introduced into the colony even if the ¾ wild strain is established by introducing only males into the existing laboratory colony (Hamm et al. 1996).

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DIFFERENTIAL GROWTH OF FALL ARMYWORM LARVAE (LEPIDOPTERA: NOCTUIDAE) REARED ON THREE PHENOTYPIC REGIONS OF WHORL LEAVES FROM A RESISTANT AND A SUSCEPTIBLE MAIZE HYBRID

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ABSTRACT

Two laboratory bioassays were conducted to determine the effect of feeding selected whorl leaf regions of a resistant and a susceptible maize, *Zea mays* L., hybrid on fall armyworm, *Spodoptera frugiperda* (J. E. Smith) growth. In one bioassay, larvae were fed fresh excised whorl leaf tissue and in the other, they were fed reconstituted diets containing ground lyophilized leaf tissue from three phenotypic leaf regions of both hybrids. Results of the two bioassays were similar. Differences in larval weights were found for those larvae fed tissue from leaf regions within and across hybrids. The largest differences among treatments were found within the resistant hybrid, thus showing that regions of the same whorl leaf differ in suitability of food source for larval growth.

Key Words: *Spodoptera frugiperda, Zea mays* L., plant resistance

RESUMEN

Se realizaron dos bioensayos de laboratorio para determinar el efecto en el crecimiento del gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), al ser alimentado con differentes partes de las hojas del cogollo de una planta de maíz, *Zea mays* L., resistente y de una susceptible. En un experimento, se alimentaron larvas con tejido fresco de hojas del cogollo y, en otro, se alimentaron con dietas reconstituídas que contenían tejido foliar liofilizado de tres regiones fenotípcas de las hojas de ambos híbridos. Los resultados de los dos bioensayos fueron similares. Se encontraron diferencias en el peso larval entre las larvas alimentadas con tejido fresco de diferentes partes de las hojas, ya sea del maíz híbrido o susceptible, y entre las larvas alimentadas con uno u otro tipo de maíz. Las diferencias más grandes entre los tratamientos se encontraron en las larvas alimentadas con hojas del híbrido resistente, mostrando así que regiones de una misma hoja del cogollo difieren entre sí en su calidad como fuente de alimento para el crecimiento larval.

Maize, *Zea mays* L., germplasm lines with leaf feeding resistance to the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and other lepidopterans [i.e., southwestern corn borer, *Diatraea grandiosella* Dyar; sugarcane borer, *D. saccharlis* (Fab.); and

European corn borer, *Ostrinia nubilalis* (Hübner)] have been released (Davis et al. 1988, Williams & Davis 1989). The primary sources of this resistance were from the Caribbean exotic germplasm Antigua Gpo. 1 and 2 and Republica Dominicana Gpo. 1.

When resistant plants are infested with fall armyworm neonates, fewer larvae survive, and those that do survive weigh less and develop slower than those on similarly infested susceptible plants (Williams et al. 1983, Ng et al. 1985). The mechanisms of resistance responsible for these adverse effects on larval survival and growth are non-preference and antibiosis (Wiseman et al. 1981, 1983). The physical and/or biochemical factors responsible for this resistance are not well understood. Williams & Davis (1997) reviewed the past research conducted to elucidate the factors responsible for the resistance and concluded that a single factor, such as a strong toxin had not been found and that resistance may be conditioned by several factors, such as leaf toughness, increased fiber, and reduced nutritional quality of the resistant plants.

Wiseman & Isenhour (1988) reported differences in weights between fall armyworm larvae feed on green and yellow whorl stage foliage from resistant and susceptible maize. Larvae fed green foliage were larger than those fed the yellow tissue (region of whorl leaf where the larvae normally feed), irrespective of whether the foliage was from a resistant or a susceptible genotype. A preliminary test conducted in our laboratory using different phenotypic regions of whorl leaves from susceptible and resistant maize hybrids as food sources for fall armyworm larvae showed similar growth patterns (F. M. D. unpublished data). We report here on a continuation and expansion of these studies to determine the larval growth responses of fall armyworm when fed on three phenotypic regions of whorl leaves from a selected resistant and susceptible hybrid.

Materials and Methods

Two laboratory bioassays, one using fresh excised whorl leaf tissue and the other lyophilized leaf powder, were conducted to study the growth responses of fall armyworm larvae fed phenotypically different regions of whorl leaves from a susceptible and a resistant maize hybrid. The hybrids selected for this study were Mp707 × Mp708 (resistant) and Ab24E × SC229 (susceptible). The inbreds used to make the resistant hybrid were developed from the Caribbean exotic germplasm (Williams & Davis 1984; Williams et al. 1990a). Plants of each hybrid were grown under field conditions in 10- row blocks. Agronomic practices recommended for our area were used to grow the maize.

The fall armyworm larvae used in these experiments were obtained from our laboratory colony. The procedures used to rear this insect on an artificial diet were described by Davis (1989).

The excised leaf tissue bioassay was begun when the plants reached mid-whorl stage. When plants were needed for feeding larvae, they were cut below the whorl. The whorl and stem portions were then placed in plastic bags by hybrid and maintained in coolers containing ice until processing in the laboratory. The inner whorl leaves were unfurled in the laboratory, and the three selected regions were excised from the leaves. The first region, referred to as green (GR) tissue, was excised from the outer, photo-exposed leaf portion about half way from the tip of the leaf to where the leaf first showed full chlorophyll content. This is a portion of the leaf that larvae normally do not feed on. The second region was within the whorl where the larvae normally feed. This region, referred to as yellow-green (YG) tissue, is just below the upper limits of the surface moisture level within the spirally rolled leaves. The third region was below the YG region. It is referred to as the yellow-white (YW) tissue because it lacks any green color. Larvae do not normally feed this deep within the whorl.

Each excised region was about 5.18 cm wide with about 5.18 cm or more between regions. Leaf mid-ribs were removed from the leaf sections prior to feeding.

Larvae were fed tissue in 8 cm dia. by 8 mm deep round, clear, plastic growth chambers (Bio Quip Products, Gardena, CA). The lid of each chamber contained 13 pinholes for exchange of gases. A 0.64 cm layer of 2% agar plus mold inhibitors (980 ml H_2O + 2.0 g agar + 0.5 g sorbic acid + 0.5 g methyl parasept) was placed in the bottom of each chamber. A piece of circular, autoclaved paper toweling was placed on top of the agar gel. After a few minutes the moisture from the agar wet the paper towel and kept it moist throughout the test period. This allowed leaf tissue placed on the towel to retain its freshness for a few days. After neonates were introduced into each chamber, a strip of autoclaved tissue paper was placed between the chamber's lid and the bottom piece. This was done to prevent neonates from escaping through the pinholes in the chamber's lid. The above description of the growth chamber is a modification of the one used by Wiseman et al. 1981 and Ng et al. 1985.

On the initial day of the experiment, two sections of tissue were placed in each chamber, one on top of the other, to provide an opportunity for the larvae to feed between leaves which is a normal condition within the whorl. Three neonates were placed on the leaf tissue within each chamber using a moistened artist brush. Three days later the number of surviving larvae was recorded and up to 2 of these larvae were removed from the chamber replicates of each treatment and weighed collectively by replication on an electronic balance. These larvae were then discarded. The remaining undisturbed larva in each chamber was fed fresh tissue as needed until the seventh day when a final weight was taken. The larvae were held under environmentally controlled conditions of 27.6°C, 50-60% RH, and a photoperiod of 16:8 (L:D).

The treatments for this bioassay were the two maize hybrids and the three whorl leaf regions of each hybrid. Treatments were arranged in a randomized complete block design with eight replications. Each treatment was represented by larvae in five growth chambers per replication.

During the same time period when whorl tissue was being processed for the excised leaf bioassay, tissue from each phenotypic region was placed in plastic freezer bags and frozen at -18°C for use in the leaf powder bioassay. Later, the frozen samples were removed from the freezer and the tissue was lyophilized, ground to a fine powder, and then returned to the freezer.

On 2 December 1997, a leaf powder diet of each leaf region for the two hybrids was prepared using the following procedure. Agar (3.5 g) was placed in a small boiler with 250 ml of water and brought to a boil. The agar/water solution was then placed into a blender. When the temperature of the solution reached 82°C, 10 g of leaf powder, 528 mg of ascorbic acid, 132 mg of sorbic acid and 132 mg of neomycin sulfate were added to the agar solution and blended for three minutes. This diet recipe is a modification of the one described by Williams et al. (1990b).

After blending, the mixture was poured into 30 ml plastic cups (25) to a depth of about 15 mm each and held under a clean-air hood for 1.5 h to cool and dry. Each cup was infested with one fall armyworm neonate. A paper-board insert cap was used to close the cup. The larvae were maintained in the same environment as described for the excised leaf bioassay.

The six leaf powder diet treatments were arranged in a randomized complete block design with five replications. Each replication consisted of five cups per treatment. Larval weights were obtained 10 and 12 days after infestation.

Mean weights for each treatment were used for statistical analysis of both bioassays. The data were subjected to analysis of variance procedure (SAS 1987) and means were separated by using Fisher's Protected Least Significant Difference test (LSD) [Steel & Torrie 1980].

RESULTS AND DISCUSSION

Excised Leaf Tissue Bioassay

Larval survival was high for all excised leaf tissue treatments. Therefore, antibiosis was not adversely affecting survival.

Significant differences in larval growth rates were clearly evident on both weigh days within and across hybrids (Table 1). Within the phenotypic leaf regions of the resistant hybrid, the order of larval size from largest to smallest was those grown on YW, GR, and YG tissue. The larvae reared on YW tissue were 4.3× and 11.4× larger than those grown on YG tissue on days 3 and 7, respectively. Larvae reared on YG versus GR tissue of the resistant hybrid did not differ in weight on day 3. However, significant differences in weight between these two leaf regions did occur on day 7. 0n this day, the larvae reared on GR tissue weighed 2.5× more than those grown on YG tissue.

Similar differences were observed among the larvae reared on the three phenotypic leaf regions of the susceptible hybrid. But, differences in weights were much less than for larvae reared on similar leaf regions of the resistant hybrid. For example, the larvae reared on YW tissue were only about 2× larger than those reared on YG or GR tissue. No significant difference was found between larvae reared on YG and GR tissues of the susceptible hybrid for both weigh days.

Larval weight comparisons across hybrids are also shown in Table 1. No significant differences were found between larvae reared on YW tissue of the resistant and the susceptible hybrid. However, significant differences were observed between larvae reared on YG tissue of the two hybrids on both weigh days. The larvae reared on YG tissue of the susceptible hybrid weighed 2.6× and 6.1× more than those reared on YG tissue of the resistant hybrid on days 3 and 7, respectively. Larvae fed GR tissue were

TABLE 1. WEIGHTS OF FALL ARMYWORM LARVAE REARED ON THREE PHENOTYPIC REGIONS OF WHORL LEAVES FROM A RESISTANT AND SUSCEPTIBLE MAIZE HYBRID ($\bar{x}$ ± SD).

			Larval weight (mg)	
			—day—	
Hybrid	Classification	Leaf[1] Tissue	3	7
Ab24E × SC229	susceptible	YW	4.8 ± 2.5[2]	275.1 ± 49.2
		YG	2.6 ± 0.6	135.3 ± 13.2
		GR	2.6 ± 0.7	155.4 ± 15.0
Mp707 × Mp708	resistant	YW	4.3 ± 0.9	251.1 ± 40.3
		YG	1.0 ± 0.2	22.1 ± 14.1
		GR	2.0 ± 1.0	55.1 ± 16.4
LSD (0.05) Values:			1.1	29.8

[1]Phenotypic regions of the whorl leaf (YW = yellow-white tissue, YG = yellow-green tissue, and GR = green tissue).

[2]ANOVA values: 3 day weights ($F = 14.71$; df = 5, 35; $P < 0.01$); 7 day weights ($F = 95.44$; df = 5, 35; $P < 0.01$).

only different in weight on day 7 when the larvae grown on the susceptible hybrid tissue weighed 2.8× more than those reared on the resistant tissue.

Leaf Powder Diet Bioassay

Significant differences in larval weights occurred among phenotypic leaf region diets within and across hybrids (Table 2). The biggest differences in weights of larvae grown on the phenotypic leaf region diets of the resistant hybrid were between YG treatment and the other two treatments. Larvae grown on GR and YW tissue diets weighed 10.6× and 6.6×, respectively, more than those reared on YG diet on day 10. About the same degree of differences occurred on day 12 among these treatments. Larvae grown on the resistant hybrid YW tissue weighed about 1.5× more than those reared on GR tissue diet of the same hybrid on both weigh days.

Significant differences in larval weights also were observed among treatment diets within the susceptible hybrid. However, these differences were of a much smaller magnitude than those within the resistant hybrid diet treatments. For example, larvae grown on YW and GR tissue diets were only about 1.4× larger than those reared on YG diet. As with the excised leaf tissue bioassay, large differences in larval weights occurred between the resistant and susceptible hybrid for those fed YG tissue diets. At both weigh days larvae grown on the susceptible YG diet weighed 7.9× more than those on YG diet of the resistant hybrid. Significant, but smaller differences were detected between GR leaf tissue diets of the two hybrids. No significant differences in larval weights were detected between those fed YW tissue diets of the susceptible and resistant hybrid.

Results from experiments using excised leaf tissue and leaf powder diet bioassays were similar. Differences in larval weights occurred among leaf tissue regions within

TABLE 2. WEIGHTS OF FALL ARMYWORM LARVAE REARED ON LYOPHILIZED LEAF POWDER DIETS FROM THREE PHENOTYPIC REGIONS OF WHORL LEAVES FROM A RESISTANT AND SUSCEPTIBLE MAIZE HYBRID ($\bar{x}$ ± SD).

			Larval weight (mg)	
			—day—	
Hybrid	Classification	Leaf[1] Tissue	10	12
Ab24E × SC229	susceptible	YW	87.7 ± 12.3[2]	136.1 ± 19.0
		YG	61.9 ± 10.7	130.5 ± 18.4
		GR	110.0 ± 15.7	240.0 ± 32.2
Mp707 × Mp708	resistant	YW	82.3 ± 19.2	167.1 ± 24.8
		YG	7.8 ± 1.4	16.5 ± 3.0
		GR	51.3 ± 7.3	120.8 ± 17.5
LSD (0.05) Values:			14.8	27.5

[1]Phenotypic regions of the whorl leaf (YW = yellow-white tissue, YG = yellow-green tissue, and GR = green tissue).

[2]ANOVA values: 10 day weights ($F = 50.12$; df = 5, 20; $P < 0.01$); 12 day weights ($F = 60.74$; df = 5, 20; $P < 0.01$).

and across the susceptible and the resistant maize hybrid. Our results were generally in agreement with those reported by Wiseman and Isenhour (1988).

The most interesting result was within the resistant hybrid whorl leaf, where suitability of food for larval growth varied from excellent (YW tissue) to very poor (YG tissue) to moderately poor (GR tissue). Thus, the resistant factor(s) must not be operative in YW tissue, but are present in YG and GR tissues. It is also interesting that larvae fed on the resistant hybrid GR tissue weighed significantly more than those fed YG tissue of the same hybrid thus, indicating a shift in intensity of resistance.

Our findings provide us with a better understanding of the susceptible and resistant whorl leaf as it relates to fall armyworm growth, and to the presence and intensity of resistance factors. Also, our results provide a new opportunity for determining the factor(s) responsible for the resistance as biophysical and biochemical characters can be compared now within the phenotypic whorl leaf regions of resistant genotypes as well as across genotype comparisons (resistant versus susceptible). This study also shows the importance of using the appropriate natural larval feeding site within the plant's whorl when conducting laboratory leaf bioassays. Using the wrong leaf region could result in incorrect conclusions.

ENDNOTE

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BIOLOGICAL DIFFERENCES BETWEEN FIVE POPULATIONS OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) COLLECTED FROM CORN IN MEXICO

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ABSTRACT

Biological characterizations of five fall armyworm populations, *Spodoptera frugiperda* (J. E. Smith) (FAW) collected from corn, Zea mays L., in Mexico, were reared and evaluated under laboratory conditions. The period from larvae to pupal stage, pupal weights, and survival rates were determined. The reproductive compatibility of adults, and the neonatal susceptibility to Endosulfan, Carbofuran and *Bacillus thur-*

ingiensis (Bt) were also evaluated. Populations from Aguascalientes, Colima, Nuevo León, Sinaloa, and Yucatán were reared on corn at 25°C. The Colima population reared on corn leaves required the least number of days to reach the pupal stage (13.04 D). Significant differences between the pupal weights of the different populations were found, ranging from 0.215 to 0.156 g. Survival rates varied from 80 to 45%, the Colima and Sinaloa populations had the highest survival. The Aguascalientes, Nuevo León and Yucatán populations were reproductively compatible as they produced progeny when paired. However, no progeny were obtained when the Colima and Sinaloa populations were paired with any other populations. The Aguascalientes, Nuevo León and Sinaloa populations tested for susceptibility to *B. thuringiensis* resulted in LC_{50} values, from 0.001 to 0.045 mg/ml). The Aguascalientes and Yucatán populations showed similar susceptibility to Carbofuran and Endosulfan insecticide with an LC_{50} ranging from 0.033 to 0.188 mg/ml, and 0.023 to 0.054 mg/ml, respectively. The Nuevo León population was the least susceptible. Results suggest that two corn FAW strains may have developed reproductive isolation due to geographic isolation. One strain formed by the Yucatán, Aguascalientes and Nuevo León populations, which are distributed along the Coastal Gulf and the geographic center of Mexico, and the other corn strain is formed by the Colima and Sinaloa populations found along the Mexican Pacific Coast, as the two strains produce no progeny when paired.

Key Words: *Spodoptera frugiperda*, reproductive compatibility, control, Mexican populations

RESUMEN

Cinco poblaciones de gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) se evaluaron en condiciones de laboratorio. Se determinaron el número de días a pupa, el peso de las pupas y su sobrevivencia. También se evaluaron la compatibilidad reproductiva de los adultos y la susceptibilidad a controles con insecticidas químicos y *Bacillus thuringiensis* (Bt). Las poblaciones de Aguascalientes, Colima, Nuevo León, Sinaloa y Yucatán se alimentaron con follaje de maíz y se mantuvieron a 25°C. La población de Colima fue la más precoz a la pupación (13.04 d). En cuanto al peso de pupa se encontraron diferencias significativas en un rango de 156 a 215 mg. El rango de sobrevivencia osciló del 45 al 80%, las poblaciones de Colima y Sinaloa presentaron la mayor sobrevivencia. Entre las poblaciones de Aguascalientes, Nuevo León y Yucatán aparentemente existió compatibilidad reproductiva; sin embargo, no se obtuvo descendencia cuando estas poblaciones se aparearon con Colima y Sinaloa, aparentemente no existió compatibilidad reproductiva entre Colima y Sinaloa, ya que al aparearlas tampoco produjeron progenie. Se determinó la susceptibilidad al Bt en las poblaciones de Aguascalientes, Nuevo León y Sinaloa, las CL50 obtenidas oscilaron entre 0.001 y 0.045 mg/ml. Las poblaciones de Aguascalientes y Yucatán presentaron resultados semejantes en cuanto a la susceptibilidad al Carbofuran y Endosulfan, con rangos en las EL50 de 0.033 a 0.188 y 0.023 a 0.054 mg/ml, respectivamente. La población de Nuevo León presentó los valores más bajos. Los resultados sugieren que existen 2 cepas o biotipos entre las poblaciones de gusano cogollero del maíz. Estas podrían haber desarrollado su aislamiento reproductivo tal vez, debido al aislamiento geográfico. Una cepa es formada por las poblaciones de Yucatán, Nuevo León y Aguascalientes localizadas en la Costa del Golfo de México y la segunda por Colima y Sinaloa, localizadas en la Costa del Océano Pacífico, ya que no produjeron progenie entre ellas.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (FAW), is a tropical insect species that is endemic to the Western Hemisphere, where it ranges from Brazil

northward, throughout Central America and North America (Mitchell 1979a). It is considered a major pest of corn, rice, and forage grasses (Pashley 1989) and is found in almost all of Mexico's agricultural areas, causing its greatest damage in the southern and eastern tropical States (Andrews 1980). Crop losses caused by the FAW range from $300 to $500 million annually in the United States of America U.S.A. (Mitchell, 1979b).

The genetic structure of FAW populations and patterns of genetic similarity among samples from different regions can provide a better understanding of migratory pathways and assist researchers in developing improved pest management strategies (Pashley, 1986). Interestingly, the study of the movement of insects in North America has greatly increased during the last 10 years (Johnson 1987). The movements of noctuids have been the focus of much of this interest, because many species are highly mobile and cause serious damage to major food and forage crops over large areas (Rabb & Kennedy 1979; Johnson & Mason 1986). Long-range movements of FAW moths from the state Mississippi to Canada on a low-level jet stream were documented on one occasion using weather maps (Johnson 1987). Many FAW moths have been detected in the Gulf of Mexico as far as 250 km from land, indicating the possibility of seasonal trans-Gulf migration between the USA and the tropics (Johnson 1987). Alternating wet and dry seasons on the east and west coasts of Central America and elsewhere in the tropics makes only one habitat available at a time. These weather fluctuations have led to the establishment, maintenance and evolution of colonizing patterns of the FAW (Johnson 1987).

Pashley et al. (1995) reported physiological differences between FAW strains. She also detected significant differences in larval development, but potential fitness consequences of adults were not examined. Pashley (1988) reported that the strains may represent one of three types of taxa: they may be biotypes in which genetic differences are due to a selectively-mediated polymorphism within a single randomly-mating species; they may be host races in the initial stages of speciation in which interbreeding is reduced due to differences in host preference, or they may be sibling species that are either capable of hybridizing to a limited degree or completely reproductively isolated.

In Mexico the differences among corn FAW populations have not been determined. Furthermore, any possible relationship between Gulf Coast populations and populations inhabiting other geographic areas of Mexico has not been established. The purpose of our research is to present information about five different FAW populations found on corn within Mexico. Two of these populations (from the states of Yucatán and Nuevo León) are found along the Gulf Coast; one population (the state of Aguascalientes) is found in the geographical Center of the Mexican Republic and two populations (the states of Colima and Sinaloa) are located along the Pacific Coast. These populations were compared by determining the biological characteristics of larvae, their susceptibility to chemical and biological insecticides, and the potential for adult interbreeding.

Materials and Methods

Insect Origin

FAW larvae for this study were collected from corn plants from different Mexican geographic areas: the Gulf Coast (Yucatán and Nuevo León) (October, 1995); the Central Highlands (Aguascalientes) (August, 1995); and the Pacific Coast (Colima and Sinaloa) (March, 1995). Four random samples from 25 consecutive plants per row were taken per geographic area (Pair et al. 1986).

Insect Rearing

The larvae were taken from the field to the laboratory and placed into individual plastic containers (100 ml), containing freshly cut corn leaves. Larvae were fed on foliage of the "Ancho" cultivar of corn until pupation. Containers were placed in the dark for 24 h to minimize phototactic responses at 25 ± 1°C and > 50% relative humidity (Quisenberry 1991; Pashley et al. 1995; Lezama-Gutiérrez et al. 1996). Corn was planted at different dates to provide fresh foliage for the larvae during the experiment. Pupae were then sexed and each group was placed in 500 ml containers with coconut fiber moistened with sterile distilled water until the emergence of adults. Adults were then placed in 30 × 30 cm cages with paper towels as oviposition substrate (Burton & Perkins 1989) and fed with 10% honey-water solution (Poitout & Bues 1974). Egg masses were collected every 24 h and placed into containers which were lined with filter paper discs and moistened with sterile distilled water until larval emergence (Whitford et al. 1992). The second generation of neonatal larvae from each population was then used to determine the number of days required to reach the pupal stage. Pupal weight, susceptibility to Endosulfan, Carboufan and *Bacillus thuringiensis* Berliner, and survival rates were recorded for each FAW population.

Microorganism Culture

The *B. thuringiensis* Kenyae serovar 07 inoculum, obtained from the "Centre de Rèférence de l'Organisation Internationale de Lutte Biologique" (Pasteur Institute), consisted of a spore powder and was kept at 4°C. The inoculum was seeded into a liquid culture medium as described by Kalfon & De Barjac (1985). Dry powder obtained had 2.4×10^8 colony forming units per g.

Development Rate of FAW Populations

Groups of 50 neonatal larvae of each FAW population were individually placed into plastic containers (100 ml) containing two pieces freshly cut corn leaves. The leaves were cut into 2-cm-long segments and fed to the larvae every 24 h. The development period of each larval instar was recorded every 24 h until reaching the pupal stage. The pupae (obtained 24 h after pupation) were weighed by an analytical balance (Ohaus Model GA22) with a sensitivity of 0.001 g. Also, the survival rate of each population was determined from neonate larvae to adult emergence. The differences in the development period of the larval stage, the pupal weight, and the survival rate were statistically analyzed (ANOVA) and the means were separated using the Student-Newman-Keuls multiple range test.

Reproductive Compatibility Between FAW populations

Fifty pupae of each FAW population were sexed and each sex group was placed in plastic containers (100 ml) with coconut fiber moistened with sterile distilled water until the emergence of adults. Plastic containers with pupae were placed into other plastic pots (1000 ml). One male and one female from different FAW populations were paired in the plastic pots (as above) with paper towels as oviposition substrate (Burton & Perkins 1989) and fed with 10% honey-water solution (Poitout & Bues 1974). Ten adult females or 10 adult males of each population were mated as single-pairs in each test. Eggs were collected daily and placed into plastic containers which were lined with filter paper discs and moistened with sterile distilled water, until larva emergence (Whitford et al. 1992). The presence of progeny from each single-pair was recorded.

B. thuringiensis Bioassays

Susceptibility of FAW larvae from Aguascalientes, Colima, Nuevo León, and Sinaloa to *B. thuringiensis* Kenyae serotype 07 was determined by LC_{50} values (Dulmage et al. 1976). *B. thuringiensis* dilution series were performed (0.003, 0.001, 0.0003 and 0.0001 mg/ml). Ten ml of sterile distilled water with Tween 0.1% of each dilution was taken. Fresh corn leaves were cut into segments (2 cm long) and inoculated by immersion for 30 s. After excess water evaporated, one piece of foliage was placed into a plastic petri dish (50 × 15 mm) lined with filter paper Whatman No. 1. The filter paper was moistened with sterile distilled water and 25 neonatal larvae per dose were used, in 2 replicates (Kalfon & De Barjac 1985; Hernández 1988). Petri dishes were placed at 25°C and kept dark for 24 h to minimize phototactic responses. Controls consisted of the same number of larvae from each FAW population fed foliage without *B. thuringiensis*. Mortality was recorded after 72 h and data were subjected to Probit analysis (Finney 1971). Maximum likelihood estimates of median lethal concentrations of *B. thuringiensis* for each FAW population were calculated. LC_{50} values were expressed as mg/ml.

Insecticide Bioassay

Based on LC_{50} values the susceptibility of the FAW neonatal larval populations from Aguascalientes, Nuevo León, and Yucatán to Carbofuran, and the FAW population from Aguascalientes and Yucatán to Endosulfan were determined (Leibee & Savage 1992). Five dilution series of each insecticide were prepared from 0.0000588 to 58.8 mg/ml of Carbofuran, and 0.0125 to 0.125 mg/ml of Endosulfan. Ten ml of sterile distilled water with Tween 0.1% of each dilution was taken. Fresh corn leaves were cut into segments (2 cm long) and inoculated by immersion for 30 s. After the excess water evaporated, one piece of foliage was placed into each plastic container (30 ml) and 5 neonatal larvae/container; 25 neonate per dose were used, in 2 replicates. Controls consisted of the same number of neonatal larvae from each FAW population fed foliage without insecticide. Mortality was recorded after 48 h and data were subjected to Probit analysis (Finney, 1971).

RESULTS

Development Rate of FAW Populations

The development rate of FAW populations from neonate to pupal stage varied between 13.04 to 16.17 d. The Colima population developed significantly faster than the other 4 populations. The pupal weights among populations varied from 0.156 to 0.215 g. Aguascalientes pupae were significantly heavier than the other four populations; with the Yucatán and Colima pupae being the lightest. The survival rate of populations from neonate until adult emergence was the highest for the Colima and Sinaloa populations (80%) while the other populations ranged between 45 to 54% (Table 1).

Reproductive Compatibility Between FAW Populations

The reproductive capacity among the different populations showed that the Aguascalientes, Nuevo León and Yucatán populations were able to produce progeny when they were paired among themselves (Table 2). On the other hand, the Colima and Sinaloa populations would not cross with any of the other populations.

TABLE 1. DEVELOPMENT RATE, PUPAL WEIGHT AND SURVIVAL RATE OF FAW POPULATIONS GROWN ON EXCISED CORN LEAVES.

FAW Populations	Number of Days to Pupation[1]	Pupal Weights (g)[1]	Survival Rate (%)
Aguascalientes	14.20 a	0.215 a	54.00
Colima	13.04 b	0.157 c	80.00
Nuevo León	15.37 a	0.168 bc	54.00
Sinaloa	15.60 a	0.178 b	80.00
Yucatán	16.17 a	0. 156 c	45.00

[1]Means within a column followed by the same letter are not significantly different (P < 0.05, LSD).

Larval Susceptibility to *B. thuringiensis*

Larvae from the Nuevo León and Aguascalientes populations were the most susceptible to *B. thuringiensis* with a LC_{50} range of between 0.001 to 0.005 mg/ml. The Sinaloa population had a significantly higher LC_{50} than the Aguascalientes and Nuevo León populations (Table 3).

Larval Susceptibility to Insecticides

There were no significant differences among the three FAW populations from Aguascalientes, Nuevo León and Yucatán to Carbufuran. LC_{50} values ranged from 0.033 to 1.36 (Table 4). Likewise, the Aguascalientes and Nuevo León populations resulted in a similar susceptibility to Endosulfan (Table 5).

DISCUSSION

Pashley (1986) reported that the FAW is composed of two genetically different strains, each of which exhibits different host specificity; one feeds on corn (genotype ABAA) and another on rice and Bermuda grass (genotype BABB). Also, no significant genotypic differences exist within the corn strain. Our results showed that, based on the development rate, the period from neonate to pupae was different between some

TABLE 2. REPRODUCTIVE COMPATIBILITY AMONG FAW POPULATIONS.

FAW Populations	Aguascalientes	Colima	Nuevo León	Sinaloa	Yucatán
Aguascalientes	+	-	+	-	+
Colima	-	+	-	-	-
Nuevo León	+	-	+	-	+
Sinaloa	-	-	-	+	-
Yucatán	+	-	+	-	+

+ = Progeny obtained; - = No progeny obtained.

TABLE 3. LC_{50} S (MG/ML) AND PROBIT ANALYSIS STATISTICS OF MORTALITY DATA FOR FAW NEONATE LARVAL POPULATIONS WHEN EXPOSED TO DIFFERENT DOSES OF *B. THURINGIENSIS* SEROVAR *KENYAE* 07.

FAW Population	LC_{50}	95% FL	Intercept	Slope
Aguascalientes	0.005	0.043 to 0.003	5.27	0.90
Nuevo León	0.001	0.005 to 0.0001	4.56	0.41
Sinaloa	0.045	0.056 to 0.037	3.38	2.49

of the corn FAW populations. The Colima population required the fewest number of days to pupation (13.4 days). Results showed that the corn foliage used to feed the larvae produced the same growth rate for all of the larval populations except for Colima, which is apparently adapted to consume this corn genotype. Isenhour et al. (1985) reported that the number of days to pupation for their FAW larvae population at 25°C ranged from 21.2 to 28.4 days when fed on corn foliage.

The pupal weight obtained from the different Mexican FAW populations showed statistical differences with ranges from 0.156 to 0.215 g. The Aguascalientes FAW population had the heaviest pupae, while the Colima and Yucatán populations resulted in the smallest pupae. Similar results were reported by Isenhour et al. (1985) for FAW larvae fed corn foliage and by Whitford et al. (1992) to larvae fed artificial diets.

The survival rate obtained from the different FAW populations showed the Colima and Sinaloa populations to be the highest. There was no relationship detected between development rate, the pupal weights, or survival rates for the 5 FAW populations studied.

The reproductive compatibility among the different corn FAW populations can be separated into two, or possibly 3, population groups. The Aguascalientes, Nuevo León and Yucatán populations were able to produce progeny when mated among these 3 populations. However the Colima and Sinaloa populations were unable to produce progeny when mated with the other three populations or with each other. Therefore, there is a good probability of seasonal trans-Gulf migration of these Aguascalientes, Nuevo León and Yucatán populations of FAW between the United States and the tropics (Johnson, 1987). These results support the hypothesis that, two or possibly 3 corn FAW strains have developed because of their geographic isolation relative to each other. Pashley (1989) reports only one corn genotype for the populations in Central America, the Caribbean, and the Southeastern USA. However, our results show that one corn FAW strain is comprised of FAW populations from Yucatán, Aguascalientes and Nuevo León and the other corn FAW strains are found in Colima and Sinaloa, along the Mexican Pacific Coast. Although Aguascalientes is more centrally located

TABLE 4. LC_{50} S (MG/ML) AND PROBIT ANALYSIS STATISTICS OF MORTALITY DATA FOR FAW NEONATE LARVAL POPULATIONS WHEN EXPOSED TO DIFFERENT DOSES OF CARBOJUAN.

FAW Population	LC_{50}	95% FL	Intercept	Slope
Aguascalientes	0.033	0.071 to 0.016	4.53	0.90
Nuevo León	1.36	7.778 to 0.238	3.99	0.47
Yucatán	0.188	0.300 to 0.096	3.4	1.26

TABLE 5. LC_{50} S (MG/ML) AND PROBIT ANALYSIS STATISTICS OF MORTALITY DATA FOR FAW NEONATE LARVAE POPULATIONS WHEN EXPOSED TO DIFFERENT DOSES OF ENDOSULFAN.

FAW Population	LC_{50}	95% CL	Intercept	Slope
Aguascalientes	0.023	0.29 to 0.018	4.07	2.65
Yucatán	0.054	0.061 to 0.048	1.58	4.66

and separated from the Gulf Coast states of Nuevo León and Yucatán by the Sierra Madre Oriental, Aguacalientes is on the eastern side of the Sierra Madre Occidental. The Sinaloa and Colima populations are separated from the other populations by the Sierra Madre Occidental and separated from each other by the Eje Neo Volcanico mountains which reach the Pacific coast in the state of Jalisco.

Our results showed that the Nuevo León and Aguascalientes populations were more susceptible to *B. thuringiensis* than the Sinaloa population. Our results showed that the Nuevo León and Aguascalientes populations have a similar susceptibility to *B. thuringiensis* as the Colima population (Hernandez 1988). These results confirm observations by Hernández (1988) that the *B. thuringiensis* serovar *Kenyae* is highly active against *S. frugiperda* larvae. The FAW larvae from the Sinaloa population, however, were less susceptible to the bacteria. These observations corroborate the findings that the *S. frugiperda* population from Sinaloa may be different from the other Mexican FAW populations.

Based on insecticide susceptibility, the Aguascalientes, Nuevo León and Yucatán populations studied were susceptible to Carbofuran, but the Nuevo León population was the least susceptible. Both Aguascalientes, and Yucatán populations resulted in a similar susceptibility to Endosulfan. More research is needed to determine the differences in susceptibility between the Pacific Coast and Gulf Coast FAW populations.

ENDNOTE

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INTEGRATED CONTROL OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) USING RESISTANT PLANTS AND ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: STEINERNEMATIDAE)

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ABSTRACT

Laboratory experiments were conducted at Tifton, GA to determine the compatibility of plant resistance with antibiosis and entomopathogenic nematodes, *Steinernema carpocapsae* (Weiser) All strain and *S. riobravis* (Cabanillas, Raulston & Poinar) for controlling prepupae of the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith). Treatments consisted of 2 nematode species as factor A, 5 diets (the regular pinto bean diet (RPBD) and RPBD diluted at the rate of 3 ml diet/2 ml water (DPBD) with added Celufil (controls), DPBD + 2.5 g of Zapalote Chico silks (ZC), DPBD + 5.0 g of ZC and DPBD + 7.5 g of ZC, as factor B, and 4 nematode concentrations (0, 2, 6 and 18 nematodes/ml) as factor C. There was a significant interaction between diets and nematode concentration. There was no significant difference in mortality of prepupae on different diets when treated with 0 or with 18 nematodes. However, when treated with 2 nematodes the mortality was significantly higher for prepupae produced on the diets containing resistant silks than for prepupae produced on RPBD or DPBD. When treated with 6 nematodes the mortality was significantly higher for prepupae produced on any of the diets containing resistant silks and the DPBD than for those produced on RPBD. Thus the effects of the resistant silks was masked by the highest concentration of nematodes, whereas, the lower levels of nematodes interacted with the resistant silks to enhance FAW mortality. This study showed that the combination of entomopathogenic nematodes and resistant corn silks could enhance the mortality of FAW prepupae and, therefore, could be useful for integrated management of this insect pest.

Key Words: *Spodoptera frugiperda,* host plant resistance, *Steinernema carpocapsae, S. riobravis,* compatibility

RESUMEN

Experimentos de laboratorio fueron realizados en Tifton, Georgia, para determinar la compatibilidad de la resistencia vegetal a través del mecanismo de antibiosis y los nemátodos entomopatógenos, *Steinernema carpocapsae* (Weiser) cepa All y *S. riobravis* (Cabanillas, Raulston y Poinar) para controlar prepupas del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith). Los experimentos se llevaron a cabo usando un diseño completamente al azar con arreglo trifactorial con tres repeticiones, 30 prepupas por repetición. Los tratamientos estuvieron constituidos por la combinación entre tres factores: dos especies de nemátodos, *S. carpocapsae* cepa All y *S. riobravis*, como

factor A; cinco dietas: la dieta regular de frijol pinto (RPBD), RPBD adicionada de celulosa (Celufil) como dietas testigo, RPBD + 2.5 g de estigmas de maíz Zapalote Chico (ZC), RPBD + 5.0 g de ZC y RPBD + 7.5 g de ZC, como factor B; y cuatro concentraciones de nemátodos: 0, 2, 6 y 18 nemátodos/ml como factor C. Los porcientos de mortalidad se registraron diariamente durante un período de 120 horas. Las concentraciones letales promedio CL_{50} se calcularon para ambas especies de nemátodos. Se encontraron diferencias significativas dentro de cada factor en todas las comparaciones. Se observó una tendencia a disminuir el número de nemátodos necesarios en ambas especies, asociados con el incremento de la concentración de estigmas de maíz. Sin embargo, *S. riobravis* mostró CL_{50} más bajas que *S. carpocapsae* cepa All. Por otro lado, las CL_{50} en las dietas portadoras de estigmas fueron más bajas que las testigos. Este estudio mostró que los nemátodos entomopatógenos y los estigmas de maíz resistente podrían incrementar la mortalidad del gusano cogollero y serían útiles en programas de manejo integrado de esta plaga.

Plant resistance (HPR) and biological control are generally considered compatible pest management strategies. The antibiotic mechanism of plant resistance offers a biologically, economically, and environmentally sound alternative to conventional pesticides for controlling the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) in corn, *Zea mays* L. (Hamm & Wiseman 1986). The potential for deleterious effects of plant quality to aid in the control of third trophic level organisms has stimulated research on compatibility of plant resistance and biological control (Bergman & Tingey 1979).

The success of natural enemies of aerial herbivorous insects can often be related to plant variety or plant chemistry (Price et al. 1980). An allelochemical called maysin, a luteolin-C- glycoside, in Zapalote Chico silks enhanced the mortality of corn earworm (CEW), *Helicoverpa zea* (Boddie) when used with a nuclear polyhedrosis virus (NPV) (Wiseman & Hamm 1993), reduced about 50% the damage by FAW, and enhanced mortality of FAW 2-fold in comparison with the susceptible check (Hamm & Wiseman 1986). From a practical standpoint, HPR is compatible with other pest management tactics where combined effects are either additive or synergistic (Hare 1992).

The FAW spends its prepupa and pupa stages in the soil, and may become infected by soil-inhabiting pathogens. Usually, soil-inhabiting insect pests are managed by the application of pesticides to soil. Interest in biological control to manage crop pests has increased because of concerns about the economic, environmental, and health costs of chemical crop protection practices, and the recognition that production systems should be both environmentally and economically sustainable (Barbercheck 1993). Nematodes in the families Steinernematidae and Heterorhabditidae show considerable potential as biological control agents against a number of soil-inhabiting pests (Kaya & Gaugler 1993). Soil is the natural habitat of a number of entomopathogenic nematodes which possess a durable and motile infective stage that can actively seek out a host insect, are virulent against a broad range of insects, and do not infect mammalian and avian fauna. Because of these attributes, as well as their ease of mass production and exemption from Environmental Protection Agency (EPA) registration, a number of commercial enterprises are producing entomopathogenic nematodes as biological control agents for inundative release (Barbercheck 1993).

Fuxa et al. (1988) reported the effect of host age and nematode strain on susceptibility of the FAW. We recently corroborated these results, but also determined that the *S. carpocapsae* All strain and *S. riobravis* were the most pathogenic against 7-day-old FAW larvae, prepupae and pupae, and that the prepupae was the most susceptible

stage (Molina-Ochoa et al. 1996). On the other hand, we also determined the amounts of resistant silks to mix into meridic diets that allow the FAW to reach the prepupae stage (Molina-Ochoa et al. 1997). It has been hypothesized that the pathogenic activity of the entomopathogenic nematodes against FAW prepupae could be enhanced by increasing the levels of plant antibiosis. Here we report the results of laboratory experiments to determine the compatibility of the HPR with varying levels of antibiosis and the entomopathogenic nematodes to control the FAW prepupae.

Materials and Methods

Insect Colony

Laboratory experiments were conducted at Tifton, Ga, in the USDA-ARS, Insect Biology and Population Management Research Laboratory (IBPMRL) during 1995. FAW used in this study were obtained from a laboratory colony at the IBPMRL, Tifton, Ga. Larvae were reared individually in plastic diet cups (30 ml) containing meridic diets.

Diets

The control groups consisted of larvae reared on a regular pinto bean diet (RPBD) (Burton 1969, Burton & Perkins 1989) and a diluted pinto bean diet (DPBD) (3 ml diet: 2 ml water) with added Celufil™. The modified diets contained 2.5, 5.0 and 7.5 g of "Zapalote Chico #2451 P(C3)" (ZC silk diets) silks per 400 ml DPBD (Wiseman & Widstrom 1986). These modified diets were selected in a previous study, because the FAW reached the prepupa stage (Molina-Ochoa et al. 1997). Diets were dispensed into 30 ml plastic cups and allowed to solidify for 2 h. The larvae were placed individually in the cups with a camels-hair brush, and held at 27 ± 2°C and 75 ± 5% RH with a photoperiod of 14:10 (L:D) (Wiseman et al. 1992). Zapalote Chico is a Mexican dent corn with silk and ear resistance to feeding by larvae of FAW and CEW (Gueldner et al. 1991, Wiseman & Widstrom 1986). When the FAW reached the prepupal stage, both controls and treatment prepupae were exposed to different concentrations of the two entomopathogenic nematodes.

Nematodes

Nematodes were obtained from Harry K. Kaya, University of California, Davis, and Grover C. Smart, Jr., University of Florida, Gainesville. Cultures of the two species of entomopathogenic nematodes used for the bioassays, *S. carpocapsae* All strain and *S riobravis*, were reared *in vivo* in sixth instar *S. frugiperda* modifying the standard rearing procedure described by Dutky et al. (1964). Nematodes were stored in tissue culture flasks and maintained in aqueous suspension at low temperature (7-10°C) and used within 1 month of collection from the White traps (White 1927).

Pathogenicity Bioassays

The Petri plate bioassay procedure was used for evaluating the virulence of both nematode species against FAW prepupae from the control and treatment diets. One prepupa was placed into each Petri dish (60 × 15 mm) containing different numbers (0, 2, 6 and 18) of infective juvenile (IJ) nematodes. The IJ of each species were suspended in 1 ml of sterile distilled water, and distributed evenly onto two pieces of 5.5

cm-diameter filter paper (Whatman No. 1) in the bottom of the Petri dish 60 min before placing the prepupa on the filter paper (Epsky & Capinera 1993). Only sterile distilled water (1 ml) was added to the controls.

The treatments were distributed in a completely randomized design with a trifactorial arrangement of treatments. The treatments were the combination of two species of nematodes, *S. carpocapsae* All strain and *S. riobravis*, as factor A; 5 diets with the 2 controls RPBD and DPBD + Celufil, and three levels of resistant silks incorporated into the DPBD (2.5, 5.0 and 7.5) as factor B; and 4 concentrations of nematodes (0, 2, 6, and 18/ml) as factor C. Each treatment had three replications, and 30 prepupae per replicate in most of the bioassays.

The Petri dishes of each replication were subsequently placed in a double plastic bag and incubated in the dark at room temperature (23 ± 2°C) to avoid dessication. Prepupal mortality was recorded every 24 h for 96 h (Epsky & Capinera 1994). The percent of cumulative mortality data was used for analysis.

Data were normalized by angular transformation prior to analysis by the General Linear Models Procedure. Significant means were separated by Least Square Means (SAS Institute 1989). Median lethal concentrations (LC_{50}) and associated statistics were estimated by the Probit analysis (Finney 1971) using POLO-PC Probit program (LeOra Software 1987).

RESULTS

There were significant differences among treatments for percent mortality ($F = 42.06$; df = 39, 80; $P < 0.0001$) of prepupae. There was no significant interaction between species of nematodes and concentration of nematodes or diets, and no significant effect due to the nematode species. A significant interaction of diets x nematode concentration was found (Table 1). The mean separation of mortalities of FAW prepupae show a positive association between all diets with the increase of nematode concentration. However, there was no significant difference in mortality of prepupae between 2 and 6 nematodes for prepupae produced on the diet containing the highest level of ZC silks (DPBD = ZC 7.5). There was no significant difference in mortality of prepupae produced on the different diets when treated with 0 or with 18 nematodes. However, when treated with 2 nematodes the mortality was significantly higher for prepupae produced on any of the diets containing resistant silks than for prepupae produced on RPBD or the DPBD + Celufil. When treated with 6 nematodes the mor-

TABLE 1. EFFECTS OF THE INTERACTION OF ZC DIETS × NEMATODE CONCENTRATIONS ON THE MEAN PERCENT OF MORTALITY OF FAW PREPUPAE.

Diet	0	2	6	18
RPBD	2.47 Aa	22.77 Ab	35.55 Ac	72.77 Ad
DPBD + Celufil	1.52 Aa	17.14 Ab	50.55 Bc	71.34 Ad
DPBD + ZC 2.5	0.50 Aa	29.33 Ab	48.00 Bc	78.00 Ad
DPBD + ZC 5.0	5.16 Aa	32.50 Bb	49.16 Bc	72.50 Ad
DPBD + ZC 7.5	0.50 Aa	42.00 Bb	49.50 Bb	76.00 Ac

Means within a column followed by the same capital letter are not significantly different. Means within a row followed by the same small letter are not significantly different ($P < 0.05$, Least Squares, SAS 1989).

tality was significantly higher for prepupae produced on any of the diets containing resistant silks and the DPBD + Celufil than for those produced on RPBD.

The results of the interaction between resistant silks and nematodes showed that fewer nematodes were required to produce 50% mortality of prepupae from the diets containing resistant silks. The LC_{50} estimated for the *S. carpocapsae* All strain ranged from 8.06 to 4.42 nematodes/ml for the control diet and the DPBD+ ZC 7.5 diet, respectively. Also, there was a reduction in the LC_{50} with the addition of resistant silks and Celufil although the 95% fiducial limits overlapped (Table 2).

The results with *S. riobravis* on FAW prepupal mortality showed a reduction in the LC_{50} from 9.39 to 4.11 nematodes, for RPBD and DPBD + ZC 2.5, respectively (Table 3). While the LC_{50s} for prepupae produced on all diets with ZC silks added were lower than LC_{50s} for prepupae produced on RPBD or DPBD plus Celufil, the 95% fiducial limits for all diets overlapped.

Discussion

This is the first report on the integration of resistant maize and entomopathogenic nematodes as a strategy to control the FAW. We selected the resistant silks of corn with meridic diets in our tests because of the well-described antibiotic relationship between FAW and CEW immature stages and maysin content in silks from the exotic variety of corn "Zapalote Chico" (Wiseman & Widstrom 1986, Gueldner et al. 1991, Wiseman et al. 1992). Maysin is an excellent antibiotic compound which inhibits the growth and development processes in both mentioned pests (Gueldner et al. 1992, Snook et al. 1994). Nematodes in the families Steinernematidae and Heterorhabditidae, along with their symbiotic bacteria, are pathogenic to insects (Poinar 1979). These entomopathogenic nematodes occur naturally in the soil and show considerable promise as biological control agents of insects (Timper et al. 1988). FAW larvae migrate from the plant to the soil and spend the pupal stage there, it is during this period that it is feasible to infect them with nematodes.

Host plants can mediate interactions between insects and biocontrol agents, increasing or decreasing the impact of the insect on the plant (Bergman & Tingey 1979, Schultz 1983). The effects of insect pathogens on their herbivorous hosts have been investigated without regard to effects of host plant on pathogen virulence (Barbercheck

Table 2. Mean lethal concentrations for *S. carpocapsae* all strain on FAW prepupae fed on diets containing different concentrations of ZC silks.

	Steinernema carpocapsae All Strain Diets				
	RPBD	DPBD + Celufil	DPBD + ZC 2.5	DPBD + ZC 5.0	DPBD + ZC 7.5
Sample Size	270	270	225	180	180
LC_{50}	8.06	7.50	7.17	7.18	4.42
95% Fiducial Limits					
Lower	5.47	5.11	4.78	4.36	2.75
Higher	12.13	11.17	10.92	11.92	6.96
Slope ± SD	1.77 ± 0.24	1.42 ± 0.22	1.14 ± 0.22	1.19 ± 0.28	1.12 ± 0.25

TABLE 3. MEAN LETHAL CONCENTRATIONS FOR *S.RIOBRAVIS* ON FAW PREPUPAE FED ON DIETS CONTAINING DIFFERENT CONCENTRATIONS OF ZC SILKS.

	Steinernema robravis Diets				
	RPBD	DPBD + Celufil	DPBD + ZC 2.5	DPBD + ZC 5.0	DPBD + ZC 7.5
Sample Size	269	252	225	180	225
LC_{50}	9.39	7.37	4.11	5.67	4.55
95% Fiducial Limits					
Lower	5.68	4.42	2.29	3.00	2.60
Higher	16.40	12.59	7.03	10.61	7.60
Slope ± SD	1.236 ± 0.22	1.765 ± 0.23	1.668 ± 0.24	1.147 ± 0.26	0.761 ± 0.22

et al. 1995). Synergistic effects between resistant plants and pathogens to control lepidopterous pests were reported by Hamm & Wiseman (1986), Wiseman & Hamm (1993) and Meade & Hare (1995). The influence of the host plant or diet on the insect susceptibility to nematode pathogenesis were previously reported by Barbercheck (1993), Epsky & Capinera (1994) and Barbercheck et al. (1995).

Our experiments were designed to determine the effects of host diet on the susceptibility of FAW prepupae to infection by entomopathogenic nematodes. The effect of host age and nematode strain or species on susceptibility of *S. frugiperda* to steinernematid and heterorhabditid nematodes was reported previously by Fuxa et al.(1988) and Molina-Ochoa et al. (1996). FAW prepupae were more susceptible (lower LC_{50}s) to *the S. carpocapsae* All strain and *S. riobravis* when fed on ZC silk diets. In the *S. carpocapsae* All strain experiment, the LC_{50}s in the least susceptible FAW prepupae, i.e., larvae which were fed RPBD and DPBD + Celufil were, 1.82-fold and 1.69-fold higher than the LC_{50} for the most susceptible treatment, i.e., DPBD + ZC 7.5.

Similar responses were observed for *S. riobravis* where the LC_{50}s were lower than those from the *S. carpocapsae* All strain. The LC_{50}s for the least susceptible FAW prepupae, those from larvae fed on RPBD and DPBD + Celufil, were 2.28-fold and 1.79-fold higher than the most susceptible, those from larvae fed on DPBD + ZC 2.5.

Cabanillas et al.(1994) recently described the nematode *S. riobravis* from soil samples in corn fields at harvest from the Lower Rio Grande Valley of Texas. Observations made in that area and the northeastern of the state of Tamaulipas, Mexico indicated that prepupae and pupae of corn earworm, *Helicoverpa zea* (Boddie) (CEW) and FAW were naturally infected by *Steinernema sp.* nematodes in about 34% and 24% of the corn fields, respectively (Raulston et al. 1992). Since then, successful results have been obtained with this species for control of CEW (Cabanillas & Raulston 1994,1995), root weevil *Diaprepes abbreviatus* L. (Schroeder 1994), and the pink bollworm *Pectinophora gossypiella* Saunders (Henneberry et al. 1995).

If the FAW larvae fed on foliage of resistant varieties of corn during the growing season, their growth would be reduced, their development time increased, and they would be exposed to parasites and predators for a longer period of time. Our data suggest that smaller prepupae entering the soil from larvae that developed on resistant plants would result in enhanced mortality by entomopathogenic nematodes and/or other biocontrol agents, preventing adults from emerging and subsequently migrating to infest other crops. Cabanillas & Raulston (1996) mentioned that delivering nema-

todes through irrigation could be a potential system for suppressing CEW populations. This proposal could also be implemented for FAW that have fed on resistant corn.

High levels of nematodes masked the effects of plant resistance on the insect. However, minimal levels of nematodes (such as 2 nematodes per insect) resulted in enhanced mortality using the combination of plant resistance and nematodes. These results support the proposal of Wiseman (1994) that the resistant cultivar is the base from which integrated pest management decisions should be made and that the integration of plant resistance with biocontrol agents, specifically pathogens and/or nematodes, should be compatible strategies for suppressing insect pest populations. Thus, the combination of resistant plants and entomopathogenic nematodes could provide crop protection that would be biologically, ecologically, economically, and socially feasible.

ENDNOTE

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ENHANCING INHERENT FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) RESISTANCE OF CORN WITH *BACILLUS THURINGIENSIS* GENES

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ABSTRACT

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a serious pest of corn, *Zea mays* L., in the southern United States. Larvae feed extensively on leaves and other parts of the plant. Germplasm exhibiting a moderate level of resistance to leaf feeding damage has been identified and released. This germplasm has been used in breeding programs for developing corn hybrids with resistance to fall armyworm and other Lepidoptera. In recent years, much effort has also been devoted to developing corn hybrids with genes from *Bacillus thuringiensis* (*Bt*) that encode insecticidal proteins. Some of these hybrids have exhibited moderate resistance to fall armyworm damage. In this investigation hybrids with both native genetic resistance and genes from *Bt* encoding insecticidal proteins were evaluated for resistance to fall armyworm in field tests and laboratory bioassays. Hybrids with both types of resistance sustained less fall armyworm damage than hybrids that had only native genetic resistance or genes from *Bt* encoding insecticidal proteins alone. Larvae that fed on hybrids with both types of resistance were significantly smaller after feeding on plants in the field or on lyophilized whorl leaf tissue in a laboratory bioassay for 10 d than larvae fed on susceptible hybrids or hybrids with only one type of resistance. Both traditional host

plant resistance and transformation of corn with genes from *Bt* provide hybrids with moderate levels of resistance, but when used together, they are complementary. Deployment of hybrids with both types of resistance should reduce losses to fall armyworm and also reduce the rate of buildups of resistance to *Bt* in fall armyworm populations.

Key words: Host plant resistance, insect resistance, maize, transgenic hybrids, transformation

RESUMEN

El gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), es una peste seria del maíz, *Zea mays* L., en el sur de los Estados Unidos. Las larvas se alimentan extensivamente de las hojas y otras partes de la planta. Germoplasma que exhibe un nivel moderado de resistencia contra daño en sus hojas se ha identificado y plantado. Este germoplasma se ha usado en programas para el desarrollo de maíz híbrido con resistencia al gusano cogollero y a otros lepidópteros. En años recientes se ha dedicado mucho esfuerzo también al desarrollo de maíz híbrido con genes de *Bacillus thuringiensis (Bt)* que codifican a proteínas insecticidas. Algunos de estos híbridos han exhibido resistencia moderada al daño causado por el gusano cogollero del maíz. En esta investigación, híbridos con resistencia genética nativa y con genes de *Bt* que codifican a proteínas insecticidas se evaluaron en cuanto a su resistencia contra el gusano cogollero en bioensayos de campo y laboratorio. Híbridos con ambos tipos de resistencia sostuvieron menos daño que híbridos que sólo tenían resistencia genética nativa o que sólo tenían genes de *Bt* que codifican a proteínas insecticidas. Las larvas que se alimentaron sobre híbridos con ambos tipos de resistencia resultaron ser significativamente más pequeñas después de alimentarse de plantas en el campo o de tejido foliar liofilizado en un bioensayo de laboratorio por 10 d comparado con las larvas que se alimentaron de híbridos susceptibles o híbridos con sólo un tipo de resistencia. La resistencia tradicional de la planta hospedera y la transformación del maíz con genes de *Bt* proporcionan niveles moderados de resistencia a los híbridos, pero cuando se usan juntos, se complementan. El desarrollo de híbridos con ambos tipos de resistencia debe de reducir la pérdida causada por el gusano cogollero al igual que debe de reducir la tasa del incremento de la resistencia al *Bt* en poblaciones del gusano cogollero del maíz.

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a major pest of corn, *Zea mays* L., in the southern United States. Larvae feed extensively on leaves and other above-ground parts of the plant. Heavy feeding can cause devastating yield losses (Sparks 1986). For 30 years USDA-ARS scientists at Mississippi State, MS have conducted research on resistance in corn to maize insect and diseases that attack it. A primary objective of this research has been the development and release of corn germplasm with resistance to fall armyworm (Williams & Davis 1997). After germplasm was released, requests for seed generally came from mainly entomologists and plant breeders with commercial seed corn companies. DEKALB Genetics Corporation has reported extensive use of this germplasm in a breeding program for developing corn hybrids with resistance to multiple species of Lepidoptera, including fall armyworm (Overman 1987, 1997).

The germplasm has exhibited only a moderate level of resistance to leaf feeding by fall armyworm (Williams & Davis 1997); therefore, efforts to identify new sources and higher levels of resistance have continued. Since the development of transformation

techniques for corn, much effort has been expended on developing corn plants expressing genes that encode insecticidal proteins isolated form the bacterium *Bacillus thuringiensis* (*Bt*) (Boulter 1993). Transgenic corn plants expressing *Bt* δ-endotoxin insecticidal proteins have been evaluated in field tests for resistance to European corn borer first brood (whorl stage attack) and second brood (reproductive stage attack) have been observed (Armstrong et al. 1995, Koziel et al. 1993). Transgenic hybrids evaluated for resistance to southwestern corn borer and fall armyworm in 1994 and 1995 at Mississippi State exhibited a high level of resistance to southwestern corn borer both years, but only a moderate resistance, especially in 1995, to fall armyworm (Williams et al. 1997).

The current investigation was undertaken to evaluate inherently resistant and susceptible corn hybrids including some with an added *Bt* gene. A primary objective was to compare the effectiveness of native genetic resistance, *Bt* insecticidal proteins, and a combination *of* the two in reducing fall armyworm in corn.

Materials and Methods

Eight proprietary experimental dent corn hybrids were provided by DEKALB Genetics Corporation, 3100 Sycamore Drive, DeKalb, IL 60115. These included two hybrids, MBR line × F-line A-Mon810 and MBR line × F-line B-Mon810, in which native genetic and *Bt* resistance were combined; the corresponding two near isogenic hybrids that lacked resistance from *Bt*, MBR line × F-line A and MBR line × F-line B; DK 591, a susceptible hybrid; DK 591-Mon810, the corresponding hybrid with resistance from *Bt* added; MBR line × M-line, a single cross hybrid with native genetic resistance in one parental inbred line; and DK 626, a susceptible hybrid. Two additional hybrids were included as checks: Mp704 × Mp707, a cross between two inbred lines with native genetic resistance, and Ab24E × SC229, a cross between two susceptible inbred lines (Williams et al. 1997).

These hybrids were evaluated for leaf feeding damage and survival and growth of fall armyworm larvae in an experiment planted at Mississippi State, MS on 17 April 1997. The experimental design was a randomized complete block with five replications of two-row plots. Rows were approximately 5 m long and spaced 1 m apart. Each plot was bordered by two rows of N7639*Bt*. Except for two hybrids that were segregating for *Bt* expression, rows were over planted at a rate of 35 kernels per row and thinned to 20 plants. The two hybrids that were segregating, MBR line × F-line A-Mon810 and MBR line × F-line B-Mon810, were planted at a double rate. Non-*Bt* expressing segregates were determined by an enzyme-linked innumosorbent assay (ELISA) strip test (GeneCheck™ √ B.t.k. Corn Lab Test Kit, Part number 70755, Monsanto Co., St Louis, MO 63198) and removed before plots were thinned. On 23 May, when plants were in the V6 to V7 stage of growth (Richie et al. 1982), plants were infested with two applications of approximately 35 fall armyworm neonates each. The larvae were taken from a laboratory colony, mixed with corn cob grits, and applied with a plastic dispenser (Mihm 1989). Leaf feeding damage was visually rated 7 and 14 d later (Davis et al. 1992) on a scale of 0, no damage, to 9, extensive damage. On 2 June, 10d after plant infestation, 10 plants were removed from each plot and dissected. The surviving larvae were counted and weighted.

Three additional rows of each hybrid were grown to provide leaf tissue for laboratory bioassays. On 29 May when plants were in the V8 stage of growth, whorls were removed, trimmed to approximately 15 cm in length, placed in plastic freezer bags, and frozen at -18°C. The frozen tissue was later lyophilized and ground to a fine powder using a laboratory mill with a 1-mm mesh screen.

Laboratory bioassays were conducted as described by Williams & Buckley (1992). Test diets were prepared form each hybrid by combining 250 ml distilled water, 2.4 g agar, and 12.5 mg gentamicin sulfate, 132 mg sorbic acid, and 528 mg ascorbic acid. The mixture was heated to 82°C while stirring, and 10 g of leaf tissue was then added. The mixture was dispensed in 10 ml aliquots into 30-ml plastic cups. Twenty cups of diet were prepared from each hybrid, and each cup was infested with one fall armyworm neonate and covered with a paperboard lid. Cups were arranged in a randomized complete block design with five replications of four cups each in an environmental chamber at 28°C and a photoperiod of 12:12 (L:D). The larvae were weighed after 10 d.

Plot means were calculated for the 7 and 14-d leaf feeding ratings, number of surviving larvae per plant, larval weight, and larval biomass (total weight of larvae per plant) for the field data. Mean larval weights were calculated from the laboratory data. An analysis of variance of data for each trait was performed (SAS Institute Inc. 1987). Hybrid means were compared by Fisher's Protected LSD Test (Steel & Torrie, 1980).

Results and Discussion

Visual leaf feeding ratings made at 7 and 14 d are given in Table 1. The numerical ratings made at 14 d were slightly higher, except for MBR line × F-line B, than those made at 7 d. The two hybrids that had both native genetic resistance and a *Bt* gene sustained the least damage. They were significantly less damaged than the corresponding near-isogenic hybrids or DK 591-Mon810, which possessed only resistance

Table 1. Fall armyworm leaf feeding damage sustained by resistant and susceptible corn hybrids evaluated at Mississippi State in 1997 ($\bar{x}$ ± SD).

Hybrid	Classification[1]	Leaf feeding damage[2]	
		7 d	14 d
MBR line × F-line A-Mon810	R × S*Bt*	3.2 ± 0.4[3]	3.6 ± 0.7[4]
MBR line × F-line B-Mon810	R × S*Bt*	3.2 ± 0.3	3.9 ± 0.5
MBR line × F-line A	R × S	6.9 ± 0.7	7.1 ± 0.7
MBR line × F-line B	R × S	6.1 ± 0.7	5.7 ± 1.3
DK 591-Mon810	S × S*Bt*	4.1 ± 0.2	4.8 ± 0.3
DK 591	S × S	8.2 ± 0.6	8.9 ± 0.2
MBR line × M-line	R × S	6.3 ± 0.8	6.8 ± 0.8
DK 626	S × S	7.2 ± 0.7	8.1 ± 0.7
Mp704 × Mp707	R × R	4.4 ± 0.4	4.7 ± 0.6
Ab24E × SC229	S × S	8.8 ± 0.3	9.0 ± 0.0
LSD (0.05)		0.7	0.8

[1]R indicates fall armyworm resistant inbred; S indicates susceptible inbred; S*Bt* indicates susceptible inbred with added *Bt* gene.

[2]Fall armyworm damage was visually rated at 7 and 14 d after plants were infested with 70 neonates each on a scale of 0 (no damage) to 9 (extensive damage).

[3]$F = 75.28$, df = 9, 36, $P \leq 0.01$.

[4]$F = 46.41$, df = 9, 36, $P \leq 0.01$.

TABLE 2. FALL ARMYWORM LARVAL SURVIVAL AND WEIGHT ON RESISTANT AND SUSCEPTIBLE CORN PLANTS AFTER 10 D IN A FIELD TEST OR LABORATORY BIOASSAY ($\bar{x}$ ± SD).

	Field[1]			Laboratory[2]
Hybrid	Larvae/plant	Larval wt. (mg)	Biomass/plant (mg)	
MBR line × F-line A-Mon810	0.7 ± 0.5[3]	2 ± 1[4]	2 ± 1[5]	93 ± 33[6]
MBR line × F-line B-Mon810	0.8 ± 0.8	2 ± 1	2 ± 1	93 ± 24
MBR line × F-line A	8.8 ± 1.5	17 ± 3	146 ± 16	406 ± 21
MBR line × F-line B	5.2 ± 3.7	12 ± 4	57 ± 33	391 ± 33
DK 591-Mon810	3.0 ± 1.6	2 ± 1	6 ± 4	179 ± 62
DK 591	13.0 ± 4.9	22 ± 5	277 ± 57	409 ± 43
MBR line × M-line	6.7 ± 3.0	11 ± 3	71 ± 26	304 ± 33
DK 626	10.3 ± 3.6	19 ± 5	189 ± 70	395 ± 29
Mp704 × Mp707	2.3 ± 1.6	7 ± 1	15 ± 10	170 ± 16
Ab24E × SC229	9.8 ± 2.7	30 ± 6	286 ± 56	400 ± 30
LSD (0.05)	2.9	4	44	44

[1]Fall armyworm larvae were recovered, counted, and weighed 10 d after plants were infested with 70 neonates each.
[2]Larvae were fed for 10 d on diets containing lyophilized leaf tissue.
[3]$F = 18.80$; df = 9, 36; $P < 0.01$.
[4]$F = 54.84$; df = 9, 36; $P < 0.01$.
[5]$F = 55.30$; df = 9, 36; $P < 0.01$.
[6]$F = 77.82$; df = 9, 36; $P < 0.01$.

from *Bt*, or Mp704 × Mp707, which had the highest level of native genetic resistance. Mp704 × Mp707 was superior to all hybrids except those with a *Bt* gene.

Fewer than one larva per plant survived to 10 d on the two MBR × S*Bt* hybrids, and the mean weight of the surviving larvae was only 2 mg (Table 2). This is consistent with the types of leaf feeding damage, primarily pinholes and small circular and elongated lesions, sustained by these hybrids. On susceptible hybrids, this type of damage is generally associated with feeding of the early instars. More than 10 larvae per plant survived on the three susceptible hybrids, DK 591, DK 626, and Ab24E × SC229. The mean weight of larvae recovered from these hybrids was approximately 23 mg.

Both larval survival and growth are components of larval biomass per plant so differences in each are reflected in this single measure of resistance. Using biomass as an indication of level of resistance, the three hybrids with a *Bt* gene did not differ from each other nor did they differ from Mp704 × Mp707, the hybrid with native genetic resistance in both parents. These hybrids were superior, however, to the three hybrids with native genetic resistance in only one inbred parent. With the exception of MBR

line × F-line A and DK 626, all hybrids with either native genetic resistance or a gene from *Bt* were significantly better than the susceptible hybrids.

Larvae reared for 10 d in the laboratory bioassay were much heavier than those that fed on plants in the field for the same period of time. The smallest larvae were those that fed on diets containing leaf tissue from hybrids with both native genetic resistance and resistance from *Bt*. Larvae fed on DK 591-Mon810, with only *Bt* resistance, and Mp704 × Mp707, with native genetic resistance in both parents, were significantly heavier than those fed on the two MBR × S*Bt* hybrids, but weighed significantly less than those fed on the R × S and S × S hybrids.

Regardless of which trait is used as a measure of resistance, the insecticidal proteins from *Bt* contribute to more resistant hybrids. In combination with native genetic resistance in one parental inbred, the *Bt* gene is even more effective. Because the one hybrid resulting from a cross between two inbred lines with native genetic resistance was significantly more resistant than those with genetic resistance in only one inbred parent, it seems reasonable to speculate that if a gene from *Bt* could be inserted into such a hybrid, the combination of native genetic resistance and *Bt* would be even more effective. It is possible that the level of resistance exhibited by the best hybrids in this investigation would be sufficient to eliminate or substantially reduce yield losses associated with fall armyworm damage in farmers' fields. Another potential benefit from deploying *Bt* genes in combination with native resistance is likely to be greater stability of the resistance.

ENDNOTE

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CUMULATIVE EFFECTS OF ANTIBIOSIS ON FIVE BIOLOGICAL PARAMETERS OF THE FALL ARMYWORM

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ABSTRACT

Results of a laboratory study showed that even low levels of resistant maize, *Zea mays* L., silks reduced fall armyworm, *Spodoptera frugiperda* (J. E. Smith) growth, extended the life cycle, and reduced fecundity in four of five generations. An intermediate level of resistance reduced growth, extended the life cycle by an average of about 4 d and significantly reduced fecundity in each of the five generations. A high level of resistant silks reduced growth significantly, extended the life cycle on an average of about 10 d per generation and reduced fecundity by almost 50% over that for the laboratory control in generations four and five. Though the estimated fecundity was not

greatly reduced in all generations, there were no apparent adjustments to the stress of various levels of resistance after five generations. Thus, fall armyworm fed on various levels of resistant silk-diets did not appear to adjust to the resistance in any of the five parameters measured after five generations.

Key Words**:** Plant Resistance; maize; corn silks; meridic diets

RESUMEN

Los resultados de un estudio de laboratorio demostraron que aún niveles bajos de resistencia en estigmas de maíz, *Zea mays* L., redujeron el crecimiento del gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), extendieron su ciclo de vida, y redujeron su fecundidad en cuatro de cinco generaciones. Una reducción de crecimiento causada por un nivel intermedio de resistencia extendió el ciclo de vida por un promedio de aproximadamente 4 d y redujo la fecundidad significativamente en cada una de las cinco generaciones. Un nivel alto de resistencia en estigmas redujo el crecimiento significativamente, extendió el ciclo de vida por un promedio de aproximadamente 10 d por generación y redujo la fecundidad por casi un 50% sobre la del control en el laboratorio en las generaciones cuatro y cinco. Aunque la fecundidad estimada no se redujo grandemente en todas las generaciones, no hubo ningún ajuste evidente a tensión causada por varios niveles de resistencia despues de cinco generaciones. Así que, aparentemente, el gusano cogollero del maíz alimentado con dietas de estigmas de varios niveles de resistencia no se ajustó a la resistencia en ninguno de los cinco parámetros medidos después de cinco generaciones.

Maize, *Zea mays* L., is one of the major sources of animal and human foods in the Americas. Maize is attacked by a variety of insect pests, but possibly the most destructive has been the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Maize is susceptible in all plant growth stages to the fall armyworm, but production is most often limited because of severe injury or complete destruction of whorl-stage plants (Wiseman et al. 1967).

Resistance in maize to larvae of the fall armyworm was reviewed by Wiseman & Davis (1979). The resistance mechanisms of antibiosis and nonpreference associated with leaf-feeding have been identified (Wiseman et al. 1981, 1983). High levels of antibiosis and nonpreference resistance mechanisms in the silks of 'Zapalote Chico', a maize cultivar from the state of Oaxaca, Mexico have since been identified (Wiseman & Widstrom 1986). A laboratory study is reported herein to demonstrate the effects of different levels of silk resistance on fall armyworm growth, development, and fecundity over five generations.

MATERIALS AND METHODS

The fall armyworm used in this study were obtained from a colony maintained at the Insect Biology and Population Management Research Laboratory, Tifton, GA. (Burton & Perkins 1989).

The maize silks were produced from a maize genotype: a resistant field maize type, 'Zapalote Chico 2451# (PC3)' (ZC) (Wiseman et al. 1983). The entry was grown in a bulk planting at Tifton, GA using agronomic practices common to the area. Open-pollinated silks of the cultivar were harvested when the silks had emerged for 2 d. Silks were excised to the ear tip, removed from the husk channel, and bulked. Silks were oven dried at 41°C. The dried silks were finely ground (1-mm screen) using a Cyclotec

TC1093 sample mill (Fisher Scientific, Atlanta). The ground silks were stored in a standard freezer (-10°C) until needed for bioassaying.

Meridic pinto bean diet (Burton & Perkins 1989) was obtained in bulk from the insect rearing section of this laboratory. For each silk treatment, the regular pinto bean diet was diluted 3ml diet: 2 ml water. Treatments for each generation were: susceptible = control, no silk/diet (no dilution); low resistance, 12.5 mg ZC silk/ml diet; intermediate resistance, 25 mg ZC silk/ml diet; high resistance, 50 mg ZC silk/ml diet; and control (regular pinto bean diet without silk) as a check for the additional generations and as a check for the original laboratory colony. Each silk treatment was mixed separately in the diluted pinto bean diet, and the diet mixtures were dispensed into 30 ml plastic cups. In addition, a new pinto bean diet control was added after the first generation and each subsequent generation from the normal laboratory fall armyworm culture (Lab Control) to detect any possible changes that might be occurring in the susceptible control from generation to generation. Larvae for generation two and subsequent generations were obtained from eggs produced by moths from each respective specific treatment. One neonate was placed in each treatment cup after the diet had cooled and solidified for about 2 h. The cups were capped, and the tests were placed in an incubator maintained at 26.7 ± 2%C, 75 ± 5% RH, and a photoperiod of 14:10 (L:D).

Treatments for each generation were arranged as a randomized complete block with 15 replications and two cups per replicate. Biological measurements recorded were weight of larvae at 9 d, days to pupation, weight of pupae (24 h old), and days to adult eclosion and estimated fecundity. Standard statistical analyses were applied to each measured parameter as a combined analysis over generations (SAS Institute 1989); means were separated by Waller-Duncan k ratio t test at k ratio = 100 and $P \leq 0.05$ (Waller & Duncan 1969).

Fecundity of fall armyworm produced from the various treatments in each generation was calculated by the regression equation developed by Leuck & Perkins (1972); where mean egg production (Y) was estimated from pupal weight (X)/replicate by the regression formula of Y = 5.33X - 423.23. Then a standard statistical analysis was applied to the treatments over generations. Means were separated by Waller-Duncan k ratio t test at k ratio = 100 and $P \leq 0.05$ (Waller & Duncan 1969).

Results and Discussion

A significant ($\underline{P} \leq 0.05$) level of resistance (treatment) by generation interaction occurred for each measured parameter. Therefore each measured parameter and generation will be discussed separately.

Weight of larvae for the first generation was significantly ($P \leq 0.05$) (df = 14; F = 107.1) different at each treatment level (Table 1). The larvae on the high resistant silk-diet weighed less than one-tenth the weight of those on the intermediate resistant silk-diet. Likewise, the development time of larvae to pupation was significantly ($P \leq 0.05$; F = 400.5) different for each treatment level with larvae that were fed the high resistant silk-diet requiring more than a week longer to pupate than the larvae fed the intermediate resistant silk-diet. Weight of pupae from larvae fed the various silk-diets showed a gradual decline ($P \leq 0.05$; F = 3.00) with increased levels of resistance, but were not distinctly separable as were 9-d weight of larvae and development times. Thus, it appears that larvae on the resistant silk-diets were able to compensate for the reduced weights at 9-d by feeding longer and attaining almost equivalent pupal weights. However, time to adult eclosion among the various diet treatments were distinctly separable ($P \leq 0.05$; F = 160.0)at each treatment level. Larvae that were fed the high resistant silk-diet required more than a week longer to reach adult eclosion than larvae that were fed the intermediate resistant silk-diet.

TABLE 1. MEAN GROWTH AND DEVELOPMENTAL TIME OF FALL ARMYWORM AFTER HAVING FED ON SUSCEPTIBLE, LOW-RESISTANCE, INTERMEDIATE AND HIGH-RESISTANCE SILK-DIETS OVER FIVE GENERATIONS.

Treatment[a]	9-d wt. (mg) larvae	Day to pupation	Wt. (mg) of pupae	Day to adult eclosion
Generation 1				
Lab C	—	—	—	—
Susceptible	537 a	11.7 a	259 a	18.1 a
Low-resistance	346 b	12.9 b	246 ab	19.6 b
Intermediate resistance	215 c	14.1 c	239 ab	21.0 c
High-resistance	17 d	21.9 d	234 b	28.8 d
SEM	21.1	0.23	6.38	0.37
Generation 2				
Lab C	302 a	13.3 a	259 a	21.7 a
Susceptible	279 a	13.4 a	258 a	21.3 a
Low-resistance	142 b	15.0 b	228 b	24.2 b
Intermediate resistance	50 c	17.4 c	238 b	25.7 c
High-resistance	24 c	20.0 d	242 b	27.9 d
SEM	13.2	0.18	5.63	0.29
Generation 3				
Lab C	380 a	12.7 a	283 a	20.5 a
Susceptible	299 b	12.8 a	250 c	20.5 a
Low-resistance	160 c	16.3 b	271 ab	23.4 b
Intermediate resistance	78 d	16.2 b	223 d	24.8 c
High-resistance	28 e	19.5 c	258 bc	26.9 d
SEM	13.1	0.14	5.56	0.18
Generation 4				
Lab C	251 a	13.5 a	282 a	21.8 a
Susceptible	253 a	13.1 a	250 b	21.4 a
Low-resistance	137 b	15.9 b	260 b	24.0 b
Intermediate resistance	35 c	18.4 c	210 c	26.3 c
High-resistance	6 d	28.3 d	162 d	36.1 d
SEM	10.2	0.34	6.88	0.32
Generation 5				
Lab C	539 a	12.4 a	285 a	20.7 a

Means within a column for each generation not followed by the same letter are significantly different (k ratio = 100, $P \leq 0.05$ [Waller & Duncan 1969]). Lab C is laboratory control larvae from the laboratory culture maintained on pinto bean diet; susceptible, diet of continuous pinto bean; low-resistance is diet of 12.5 mg dried resistant silks/ ml dilute pinto bean diet; intermediate-resistance is a diet of 25 mg dried resistant silks in dilute pinto bean diet; high resistance is a diet of 50 mg dried resistant silks in dilute pinto bean diet. SEM = standard error of the mean was based on pooled error mean square in the analysis.

TABLE 1. (CONTINUED) MEAN GROWTH AND DEVELOPMENTAL TIME OF FALL ARMYWORM AFTER HAVING FED ON SUSCEPTIBLE, LOW-RESISTANCE, INTERMEDIATE AND HIGH-RESISTANCE SILK-DIETS OVER FIVE GENERATIONS.

Treatment[a]	9-d wt. (mg) larvae	Day to pupation	Wt. (mg) of pupae	Day to adult eclosion
Generation 5				
Susceptible	292 b	13.2 b	227 b	21.1 a
Low-resistance	108 c	16.1 c	275 a	23.5 b
Intermediate resistance	46 d	17.4 d	212 c	25.9 c
High-resistance	10 e	24.4 e	183 d	31.8 d
SEM	12.7	0.26	5.38	0.30

Means within a column for each generation not followed by the same letter are significantly different (k ratio = 100, $P \leq 0.05$ [Waller & Duncan 1969]). Lab C is laboratory control larvae from the laboratory culture maintained on pinto bean diet; susceptible, diet of continuous pinto bean; low-resistance is diet of 12.5 mg dried resistant silks/ ml dilute pinto bean diet; intermediate-resistance is a diet of 25 mg dried resistant silks in dilute pinto bean diet; high resistance is a diet of 50 mg dried resistant silks in dilute pinto bean diet. SEM = standard error of the mean was based on pooled error mean square in the analysis.

For generation two, the weight of larvae at 9-d that fed on the intermediate and high resistant silk-diet were not significantly ($P \leq 0.05$) different (df = 14,4; F = 94.5) (Table 1), though the larvae fed on the intermediate resistant silk-diet were more than twice the weight of those fed on the high resistant silk-diet. The development time of larvae to pupation among the various resistance diet levels was significantly ($P \leq 0.05$; F = 260.1) longer than for larvae that were fed on the two check diets. Development time for larvae that were fed on the high resistant silk-diet were only 2.5 d longer than for larvae that were fed on the intermediate silk-diet. As before, it appeared that the longer the larvae fed on a diet before pupation, compensation of weight occurred ($P \leq 0.05$; F = 5.58). Again, the development time to adult eclosion was longer ($P \leq 0.05$; F = 87.8) as the level of resistance in the silk-diets was increased.

Nine d weight and development time of larvae fed the various resistant silk-diets during generation three were significantly ($P \leq 0.05$) ($P \leq 0.05$; F = 129.0) less than those that were fed the susceptible checks (Table 1). Larvae that were fed the high resistant silk-diet weighed significantly less than those fed the intermediate silk-diet. Weight of pupae for larvae fed the various diets was similar to those in generations one and two ($P \leq 0.05$; F = 16.7). Development time to adult eclosion was clearly separable ($P \leq 0.05$; F = 238.0) among the various levels of resistant silk-diets. Larvae that were fed on the high resistant silk-diet required the longest time before eclosion.

For generations four and five clearer separations occurred among the various treatment levels for 9-d weight of larvae, development time of larvae, weight of pupae and days to adult eclosion (Table 1). The weight of larvae at 9-d that were fed the high resistant silk-diets weighed significantly ($P \leq 0.05$) (df = 14, 4) (F = 130.8, 298.5) less than larvae fed the intermediate silk-diets for both generation four and five. Weight of pupae were also significantly less for larvae feeding on the high resistant silk-diet for both generations ($P \leq 0.05$; F = 45.0, 62.8). Likewise, days to adult eclosion was similar for generation four and five in that larvae feeding on the high resistant silk-diet ($P \leq 0.05$; F = 300.8, 176.8) required the longest to emerge as adults.

From the data reported here on fall armyworms maintained on various levels of resistant silk-diets, it appears that the larvae were unable to adjust to the resistant

TABLE 2. ESTIMATED MEAN EGG PRODUCTION OF FALL ARMYWORM ADULT FEMALES REARED FROM LARVAE ON VARIOUS LEVELS OF RESISTANT SILK-DIETS.

	Generation				
Treatment[1]	1	2	3	4	5
Lab C	—	961 a	1082 a	1081 a	1004 a
Susceptible	960 a	952 a	911 c	912 b	788 b
Low-resistance	886 ab	793 b	1021 ab	965 b	1040 a
Intermediate resistance	853 ab	848 b	764 d	698 c	709 c
High-resistance	823 b	867 b	951 bc	441 d	592 d
SEM	35	30	30	36	29

[1]Fecundity per female was based on the regression equation of Leuck & Perkins (1972) where ^Y = 5.33X - 423.23 and X = average weight of pupae/replicate/treatment. Means within a column for each generation not followed by the same letter are significantly different (k ratio = 100, $P \leq 0.05$ [Waller & Duncan 1969]). Lab C is laboratory control larvae from the laboratory culture maintained on pinto bean diet; susceptible, diet of continuous pinto bean; low-resistance is diet of 12.5 mg dried resistant silks/ ml dilute pinto bean diet; intermediate-resistance is a diet of 25 mg dried resistant silks/ml dilute pinto bean diet; high resistance is a diet of 50 mg dried resistant silks/ml dilute pinto bean diet. SEM = standard error of the mean was based on pooled error mean square in the analysis.

silks even after five generations. Differences were detected between the two checks (generation two-five) among the various parameters measured in only six of sixteen comparisons. Three of these instances were for weight of pupae where compensation appeared when larvae fed longer on the various resistant diets. However, only one difference between the two checks occurred for development time of larvae and that was in generation five. The mean separations for the various parameters measured became more distinct for larvae that were fed the resistant silk- diets as the number of generations increased.

The estimated fecundity of fall armyworm fed the various silk-diets varied among treatment levels (Table 2) ($P \leq 0.05$). It appeared that the intermediate and high resistant silk-diets that were fed to larvae reduced the fecundity of females emerging from these diets, especially in generations four and five ($F = 16.7, 62.8$). Though the estimated fecundity was not greatly reduced, there certainly was some impact on fecundity to the higher levels of resistance as compared to earlier generations.

In summary, in each generation, weight of larvae was significantly lower and development time was significantly longer for larvae that were fed the high-resistant diets than for larvae fed the intermediate silk-diet and especially for larvae fed the other treatment diets. Estimated fecundity was reduced, especially for generation four and five for larvae that were fed the high resistant silk-diet. Though the estimated fecundity was not greatly reduced in all generations, there were no apparent adjustments to the stress of resistance occurring in any of the five parameters measured after five generations. Thus, fall armyworm fed on various resistant levels of silk-diets did not appear to adjust to the stress of the various levels of resistance after five generations.

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A REVISION OF THE GENUS LYGOFUSCANELLUS (HETEROPTERA; LYGAEOIDEA; RHYPAROCHROMIDAE)

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ABSTRACT

The genus *Lygofuscanellus* Scudder is redefined and the status of the stridulitrum as a taxonomic character discussed. A key to species is included. Two new species, *L. elegans* and *L. ozophoroides*, are described from South and Central America. *Ozophora baliocoris* Slater is transferred to *Lygofuscanellus*. The type species of *Lygofuscanellus* (*alboannulata* Champion) is redescribed and additional distributional data given. Dorsal views of *L. alboannulatus* and *L. elegans* are included.

Key Words: *Lygofuscanellus*, *Ozophora*, Lygaeoidea, stridulatory structures, Neotropical

RESUMEN

Se redefine el género *Lygofuscanellus* Scudder y se discute la condición del stridulitrum como carácter taxonómico. Se incluye una clave para las especies. Se describen dos especies, *L. elegans y L. ozophoroides*, de Centro y Sudamérica. Se transfiere *Ozophora baliocoris* Slater a *Lygofuscanellus*. La especie tipo de *Lygofuscanellus* (*alboannulata* Champion) se redescribe y se presentan datos adicionales de distribución. Se incluyen ilustraciones dorsales de *L. alboannulatus* y *L. elegans*.

The genus *Lygofuscanellus* was established by Scudder (1962) in the Cleradini (*sensu* Scudder) for *Pamera alboannulata* Champion (1913) who described the species from Costa Rica. Scudder related the genus to *Ozophora* Uhler from which he stated (without comparative information) that it differed in color and the shape of the head and pronotum. Actually none of these features distinguish *Lygofuscanellus* from *Ozophora* which it closely resembles. Scudder's comments that the lateral pronotal margins are carinate and that the pronotum lacks a transverse impression are not completely true. In recent literature the carinate condition of the lateral pronotal margins has been restricted to a sharp knife-like edge to distinguish it from the raised but obtuse margins found in most *Ozophora*. Species of *Lygofuscanellus* are like most species of *Ozophora* in this feature. The really distinctive feature mentioned by Scudder is the presence of a distinct lunate stridulitrum on abdominal sterna two, three and four. Ashlock & Slater (1982) recognized the genus on the basis of this abdominal stridulitrum.

The recognition of genera on the basis of the presence or absence of an abdominal stridulitrum is one of questionable validity, but is adopted here as a matter of convenience more than a belief that such a feature will prove in the long run to be of synapomorphic significance. One must recognize that a similar condition occurs in at least three genera of myodochine lygaeoids (*Ligyrocoris*, *Froeschneria* and *Pseudopamera*) in the western Hemisphere and in *Afrovertanus* in Africa. These genera were all recognized by Harrington (1982) as distinct taxa.

The whole question of monophyly based on the occurrence of similar stridulatory structures in the Lygaeoidea is a perplexing one. For example, there is a wing stridulitrum along the edge of the corium in members of several tribes. Even more striking is the possibly convergent head stridulitrum in the pamphantine genus *Cattarus* and in the new world Colobathristidae.

My decision to recognize *Lygofuscanellus* as a distinct genus for the present is based on the already complex nature of the genus *Ozophora*. Placing the species treated here in *Lygofuscanellus* within *Ozophora*, while defensible, would not add to knowledge of the relationships of species within the latter since the species discussed here appear to form a monophyletic group whether they are recognized as a distinct genus or as a unit within *Ozophora*.

There is little doubt but that these species are very closely related to species of *Ozophora* which they resemble in size, shape, type of fore femoral spines, color patterns and most other structural details. Indeed Slater (1983) placed *Ozophora baliocoris* in that genus despite his recognition of the presence of an abdominal stridulitrum.

Thus while most of the features of *Lygofuscanellus* are shared with *Ozophora* the third antennal segment is usually enlarged and fusiform in members of this genus suggesting monophyly.

Almost nothing is known of the biology of any of the species. Champion (1913) reported his specimens from bromeliads and there are, as noted below, records of interception on orchids and bananas. However there are no actual field observations.

The species are rare in collections. I have examined thousands of specimens of *Ozophora* from light traps as well as having seen extensive concentrations of these insects in the field yet the few specimens of *Lygofuscanellus* listed below is all of the material that has come to my attention. To illustrate this is the collection of three specimens of *Lygofuscanellus alboannulatus* at light at the Simla Biological station by Dr. Michael Emsley. Emsley collected extensively at light at this locality as have many other collectors including those concentrating on lygaeoids such as Dr. Baranowski (U. Florida, Homestead) and myself, yet no other specimens have come to hand. Dr. Baranowski's extensive collecting on Trinidad did not result in the collection of a single specimen. All of this suggests that these may not be ground living lygaeoids and may well occur in specialized habitats.

All measurements are in millimeters.

Key to the Species of *Lygofuscanellus*

1. Head, pronotum and scutellum completely black or dark chocolate brown, unicolorous . . . . . *alboannulatus* (Champion)

1'. Pronotum with a distinct pattern of dark and light longitudinal stripes on posterior pronotal lobe; scutellum with a pair of light spots or stripes present 2

2. Pronotum with a light median line running through dark central ovoid stripe of posterior pronotal lobe . . . . . *baliocoris* (Slater)

2'. Median area of posterior pronotal lobe with a complete dark stripe, lacking a median pale line, a broad median dark stripe present on posterior pronotal lobe. . 3

3. Third antennal segment dark chocolate brown, strongly swollen and fusiform; length of pronotum less than one and one fourth times length of scutellum . . . . . *elegans* new species

3'. Third antennal segment terete, not conspicuously fusiform, usually reddish brown; length of pronotum more than one and one third times length of scutellum. . . . . *ozophoroides* new species

Lygofuscanellus alboannulatus (Champion)
Fig. 1

Pamera alboannulata Champion 1913: 6-7.
Lygofuscanellus alboannulatus Scudder 1962: 988.

(Redescription of Lectotype)

Body color chiefly dark chocolate brown to black including entire head, pronotum, scutellum, clavus, an elongate stripe on corium adjacent to claval suture, a complete transverse fascia (with a large white spot near inner angle), apical corial margin, a large apical corial macula and entire membrane (latter lacking an apical light macula or stripe). Remainder of corium and apex of scutellum white. Dark markings on corium causing development of a large bluntly triangular white subapical corial patch. All femora reddish brown, contrasting with pale yellow tibiae and tarsi. First, third and apical two-thirds of antennal segment four reddish brown to chocolate brown. Second antennal segment yellow. Basal one-third of fourth antennal segment with a conspicuous white annulus. Dorsal and pleural surfaces dull, former bearing numerous elongate hairs. Pronotal punctures on posterior lobe obscure, those on hemelytra large and darkened.

Head non-declivent. Eyes large set well away from anterior margin of pronotum. Vertex slightly convex. Tylus attaining middle of first antennal segment. Length head 0.90, width 0.94, interocular space 0.46. Pronotum with lateral margins deeply concave, transverse impression shallow but complete, posterior lobe moderately elevated above anterior lobe, humeral angles rounded, posterior margin deeply concave. Length pronotum 1.08, width 1.40. Scutellum moderately swollen on distal half. Length scutellum 0.86, width 0.68. Length claval commissure 0.72. Midline distance apex clavus-apex corium 1.20. Midline distance apex corium-apex membrane 0.80. Metathoracic scent gland auricle finger-like, angled posteriorly. Evaporative area poorly defined, occupying inner half of metapleuron, distally truncate. Fore femur with 3 large pediculate spines and a smaller more proximally placed spine (left femur appearing to have only two large spines). Labium apparently extending to or between mesocoxae. Length labial segments I 0.62, II 0.62, III 0.46 (approx.), IV (obscured). First antennal segment stout, second segment slender, terete, third segment moderately fusiform. Length antennal segments I 0.48, II 1.40, III 1.34, IV 1.34. Total body length 5.57.

The above description is taken from the male lectotype in the Natural History Museum London. It is a male bearing the following labels. 1. round label with red border "type:". 2. A handwritten label "Pamera alboannulata Ch." 3. Pointed label "♂" 4. "Orosi Costa Rica ex C. Picado" 5. "Found in Bromeliads" 6. "1913-83" 7. a round label with a purple border "Lectotype" 8. "Pamera alboannulata Champion, G. G. E. Scudder LECTOTYPE".

There is very little variation in the material listed below. Some specimens have a more strongly fusiform third antennal segment than do others and there is variation in the size of the white annulus on the fourth antennal segment. One Trinidad specimen has the right antenna oligomerous.

This species is readily recognized by the much darker coloration than any of the others. In all other species the posterior pronotal lobe has elongate stripes and "loops" whereas it is completely dark in this species.

Distribution: Originally described from Costa Rica and previously known only from there. Specimens examined: GUATEMALA: intercepted Philadelphia 29.VI.1936 "ship light socket". (USNM). PERU: intercepted Miami, Fla. 7.II.1961 "with orchid plant" (USNM). FRENCH GUIANA: Montagne des Singes nr. Kourou, 3.VI.1986 (Riley & D.A. Rider) (at light) (RIDER coll.); TRINIDAD: Simla Biol. Stat. (M. Emsley) (at light) (JAS, RMB).

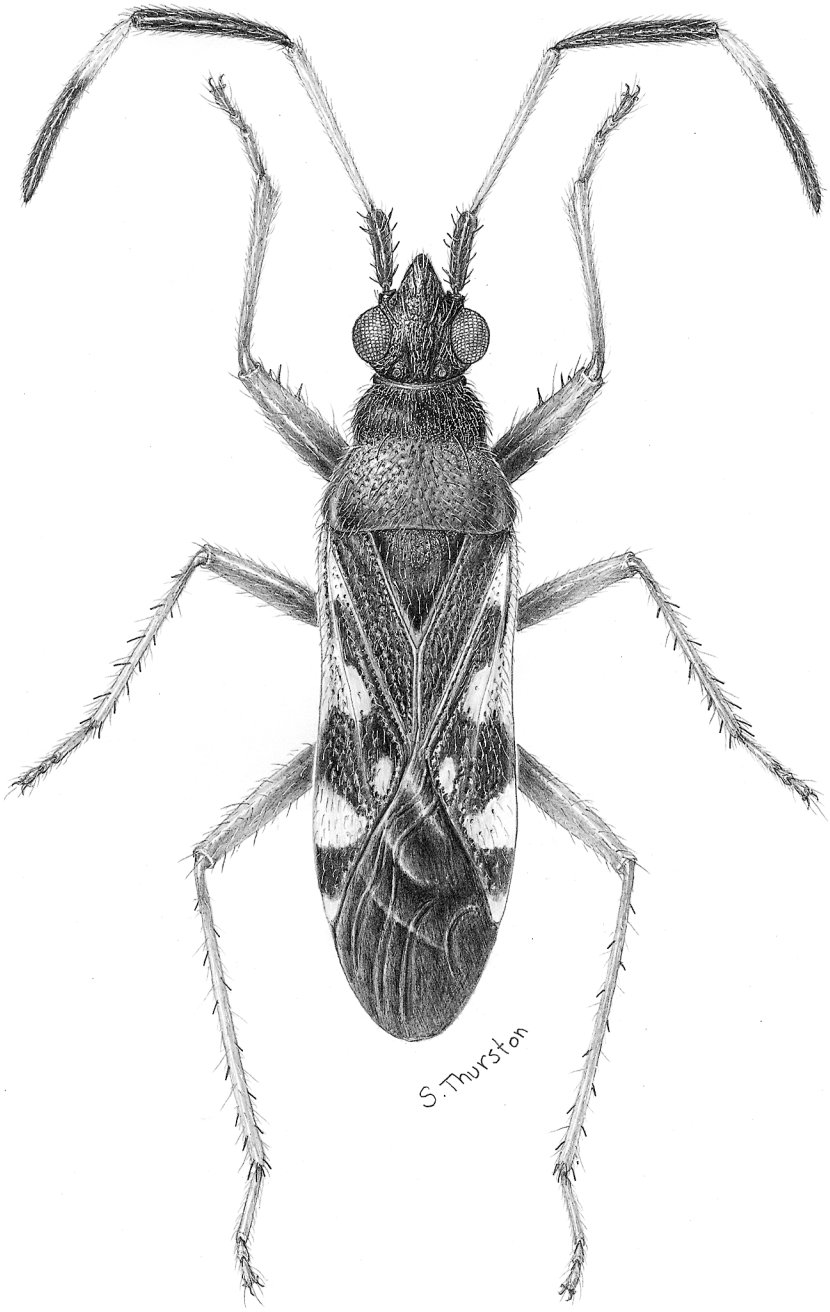

Fig. 1. *Lygofuscanellus alboannulatus* (Champion). Dorsal view.

Lygofuscanellus elegans **new species**
Fig. 2

Head and anterior pronotal lobe black, with anterior collar yellow. Dorsal surface otherwise extensively marked with dark brown on a yellow background. Posterior pronotal lobe with a broader dark brown median stripe and a pair of sublateral loops, leaving lateral and posterior margins of posterior lobe, including humeral angles, pale. Scutellum dark brown with exception of a pair of small, pale yellow, sublateral spots and a white apex, becoming gray pruinose on anterior half. Clavus brown but with central portion of claval vein pale. Corium with a broad, complete dark transverse fascia, an elongate dark streak between medius and radius and a large subapical dark patch which leaves a conspicuous distal white macula to corium. Apical corial margin dark brown on anterior 4/5. Membrane dark fumose, lacking an apical pale area, veins in part pale. Antennal segments one and two bright red-brown, third segment and distal half of segment four chocolate brown, almost black, basal half of segment four a strongly contrasting white. Fore femur, distal halves of middle and hind femora and fourth labial segment reddish brown. Dorsal surface with numerous upstanding hairs. Dorsal and pleural surfaces entirely dull. Patches of gray pruinosity present laterally near transverse impression of pronotum and near anterior collar. Head granulose. Anterior pronotal lobe with a few inconspicuous punctures. Posterior pronotal lobe, clavus and corium with conspicuous brown, but well separated, punctures.

Head with vertex moderately convex, tylus extending anteriorly only over basal third of first antennal segment. Eyes large, sessile, set well away from anterior margin of pronotum. Length head 0.92, width head 1.00, interocular space 0.52. Pronotum with anterior collar well defined and punctate; lateral margins deeply concave; a complete transverse impression present. Posterior pronotal lobe considerably elevated above anterior lobe; humeral angles rounded. Length pronotum 1.20, width 1.74. Scutellum slightly swollen on posterior half. Length scutellum 1.08, width 0.86. Length claval commissure 0.90. Midline distance apex clavus-apex corium 1.32, midline distance apex corium-apex membrane 1.20. Metathoracic scent gland auricle slender, finger-like, angled caudo-laterad. Evaporative area occupying inner three-fourths of metapleuron, truncate distally, poorly differentiated. Fore femur moderately incrassate, armed below on distal half with three sharp, pediculate spines. Labium extending between mesocoxae. first segment remote from base of head. Length labial segments I 0.30, II 0.80, III 0.56, IV 0.38. First antennal segment stout, second slender and terete, third swollen and fusiform, thicker than segment IV. Length antennal segments I 0.78, II 1.52, III 1.40, IV 1.54. Total body length 6.74.

Holotype: ♀. ECUADOR: Intercepted New Orleans 23.IV.1958 (Shiff-tlc (sp?) ("on bananas"). In National Museum of Natural History (USNM).

This specimen has been held for many years in the hope that additional specimens with more definitive locality data would become available.

Lygofuscanellus ozophoroides **new species**

Very similar to *L. elegans* in color and structure, differing in having elongate yellowish dashes on scutellum, lacking dark brown color markings on hemelytra anterior to the dark brown transverse fascia (although clavus infuscated) and by having the third antennal segment red-brown, concolorous with antennal segment two and linear, not swollen nor noticeably fusiform, and much paler than distal half of antennal segment four.

Length head 0.94, width 1.06, interocular space 0.44. Length pronotum 1.22, width 1.80. Length scutellum 0.88, width 0.90. Length claval commissure 0.92. Mid-

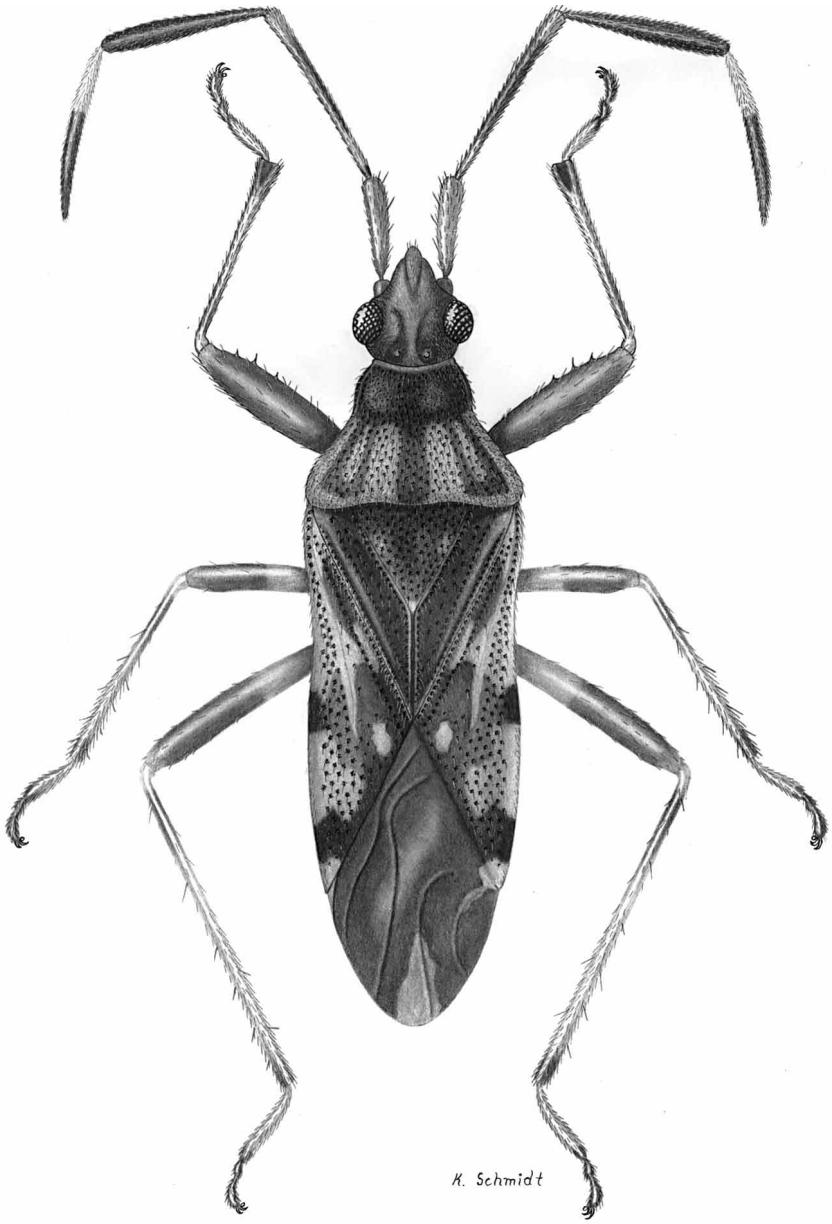

Fig. 2. *Lygofuscanellus elegans* new species. Dorsal view.

line distance apex clavus-apex corium 1.28. Midline distance apex corium-apex membrane 1.14. Length labial segments I 0.88, II 0.86, III 0.62, IV 0.38. Length antennal segments I 0.80, II 1.68, III 1.42, IV 1.90. Total body length 7.14.

Holotype: ♀. MEXICO: Chiapas, Bonompak, 21.V.1980 (E. Barrera). In Instituto de Biologia, U. Mexico (UNAM).

Paratypes: COSTA RICA: 3♀♀. Prov. Puntarena, Osa Peninsula, Tropical Science Center 5 km W. of Rincon de Osa, 26.VIII.1971 (D. J. Pool) (black light trap). PANAMA:1♀. Barro Colorado I. 5.XI.1973 (H. Wolda). 1 ♀. BELIZE: ("British Honduras") Toledo District Columbia For. Sta. 28.VII.1968 (W. L. Hasse) (black light trap). GUATEMALA: (no abdomen) Cayuga XII-1915 (Wm. Schaub). In Texas A. & M. University; RMB and JAS collections.

Lygofuscanellus baliocoris (Slater) **new combination**

Ozophora baliocoris Slater 1983: 6-7.

Slater (1983) described this species in *Ozphora* despite recognizing the presence of an abdominal stridulitrum. However, since *Lygofuscanellus* is recognized here as a valid genus in this paper this species must be placed there.

It is a smaller species than the others and readily distinguishable by the pale yellow median line that extends through the pronotum and scutellum, by a white calloused macula on either side of the midline immediately behind the transverse pronotal impression, by the spotted femora, incomplete dark transverse corial fascia, reddish brown anterior pronotal lobe and by the large white macula at the end of the membrane of the front wing.

The species was described from Mexico, Panama, Costa Rica and Ecuador. I have seen additional specimens from Panama and Mexico. It is apparently a widespread species, but like the other members of the genus, is scarce in collections.

ACKNOWLEDGMENTS

I am indebted to Mr. Mick Webb (Natural History Museum, London) for the loan of the lectotype of *Pamera alboannulata*; to Dr. Carl Schaefer (U. Connecticut) for assistance with the literature; to Dr. R. M. Baranowski (U. Florida, Homestead); Dr. R. C. Froeschner (National Museum of Natural History (USNM); Dr. Harry Brailovsky (UNAM, Mexico); Dr. Joseph Schaffner (Texas A. & M. University); Dr. Dodge Engleman and the late Dr. P.D. Ashlock for the loan and gift of material, and to Mrs. Kathleen Schmidt and Mr. Steven Thurston (both formerly U. of Connecticut) for execution of the illustrations.

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EFFECT OF SOIL MOISTURE ON DEVELOPMENT OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

We conducted trials to determine conditions of soil moisture required to optimize production of adults of *Diaprepes abbreviatus* (L.) in a laboratory colony. Larvae of *D. abbreviatus* were reared on a commercially available artificial diet and then placed in soil with water content ranging from 20 to 80%. Optimal moisture content of soil for pupation was determined to be 60 ± 10% by weight. When 68-d-old larvae were transferred from artificial diet to soil with these moisture levels and constant temperature (25°C), mean (± SEM) development time of *D. abbreviatus* from neonate to pupa was 126 ± 2.3 d (n = 47). For all pupae, the time required for pupation did not vary with soil moisture. When 68-d-old larvae were taken from diet and placed in soil, the proportion that pupated varied with moisture content. Low (20-40%) and high (80%) moisture content resulted in increased mortality, and fewest larvae pupated within the low range. The mean ± SEM number of days to pupation of 68-d-old larvae was 58.2 ± 2.3 (n = 47). Older (180 d) larvae reared on diet pupated over a greater range of moisture treatments (30-70%) and were adversely affected only by the highest (80%) and lowest (20%) treatments. Mean ± SEM time to pupation (38.4 ± 1.9 d, n = 54) did not vary for 180-d-old larvae kept at 30-70% moisture content.

Key Words: Development, soil moisture, pupation, citrus root weevil

RESUMEN

Realizamos ensayos para determinar las condiciones de humedad de suelo requeridas para optimizar la producción de adultos de *Diaprepes abbreviatus* (L.) en una colonia de laboratorio. Para determinar las condiciones óptimas de humedad para la pupación, se criaron larvas de *D. abbreviatus* con una dieta artificial comercial, y se colocaron en suelo con un contenido de agua entre 20 y 80%. El contenido óptimo de humedad del suelo fue de 60 ± 10% por peso. Cuando las larvas de 68 días de edad fueron transferidas de la dieta artificial al suelo en este rango de humedad y con temperatura constante de 25°C, el promedio (± error estandard del promedio) del tiempo de desarrollo de *D. abbreviatus* criada desde neonata hasta pupa fue de 126 ± 2.3 d (n = 47). Para las pupas, el tiempo requerido para la pupación no varió con diferentes tratamientos de humedad del suelo. De las larvas transferidas a los 68 d de dieta a suelo, la proporción que empupó varió con la humedad del suelo. Bajo (20-40%) y alto (80%) contenido de humedad causaron un incremento de mortandad; menos larvas empuparon en los tratamientos de baja humedad. El promedio del número de días hasta la pupación de larvas de 68 d fue de 58.2 ± 2.3 (n = 47). Las larvas de 180 d criadas con una dieta artificial empuparon bajo condiciones más amplias de humedad (de 30 a 70%) y su pupación fue menos solamente en el tratamiento más húmedo (80%) y más seco (20%). El promedio del tiempo a empupación (38.4 ± 1.9 d, n = 54) no varió para larvas de 180 d mantenidas en los tratamientos de 30 a 70% de humedad del suelo.

The root weevil *Diaprepes abbreviatus* (L.), is increasing in importance as a pest of Florida citrus and ornamentals as its range expands throughout central and southern portions of the state. Twenty counties currently are listed as infested by the Florida Department of Agriculture and Consumer Services. This weevil is remarkable for its wide host range and the severity of damage inflicted on individual citrus trees (Simpson et al. 1996, Schroeder & Sutton 1977). Timely detection of larvae and adults of *D. abbreviatus* is difficult and few control options are available. A colony of *D. abbreviatus* was established at the U.S. Horticultural Research Laboratory at Orlando, FL in 1992. The rearing method has developed empirically and little is understood about the effect of temperature and soil moisture content on larval developmental rate and pupation. Developmental time under current colony conditions is highly variable making rearing costly and inefficient. Under colony conditions, pupation is irregular and occurs between 3 and 18 months after egg eclosion.

Beavers (1982) reported successful rearing of *D. abbreviatus* on an artificial diet and estimated the total development time to be approximately 1 year, as did Wolcott (1934). Wolcott felt pupation of *D. abbreviatus* occurred in the spring, presumably with the onset of rains based on observations of Barrow (1924). His observations that eggs occur in the field throughout the year argue for a flexible developmental plan wherein developmental time can vary from 6 months to more than a year. Beavers & Selhime (1986) also suggested increased rainfall precedes peaks in adult numbers. Recently, speculation has focussed on the possibility of genetic variability in developmental rate and time to pupation. However, to assess genetic variation in developmental traits, it will first be necessary to establish uniform, optimal rearing conditions to eliminate environmental effects on development and pupation.

Identification of host plant resistance in citrus and near-citrus relatives relies on bioassays involving larval feeding (Shapiro & Gottwald 1995, Shapiro et al. 1997). Efforts to measure aspects of plant-insect interactions and to assess efficacy of control options for this increasingly important pest will benefit from a definition of the environmental parameters required for development and pupation. Tarrant & McCoy (1989) characterized the effect of temperature on 3 genera of root weevils that attack citrus, but did not include *D. abbreviatus*. A brief report by Schroeder (1987) alluded to the potential for inducing pupation in laboratory-reared *D. abbreviatus* by transferring larvae from diet to soil. We report here the results of a controlled study of the response of larval *D. abbreviatus* to conditions of soil moisture.

Materials and Methods

Rearing.

All stages of *D. abbreviatus* were reared at the U.S. Horticultural Research Laboratory, Orlando, FL. Eggs were collected from caged adults on wax-paper strips (Wolcott 1933) and allowed to eclose in plastic containers. Diet for larval development was prepared as follows: 40 liters of water were combined with 725 g agar and heated to near boiling. While stirring, 9.5 kg of commercially prepared insect diet [product no. F1675, Bio-Serv, Inc., Frenchtown, NJ, similar to that developed by Beavers (1982)] were added to the water/agar mixture, mixed, and heated to between 200 and 230°C. Methylparaben (9 g dissolved in 10 ml 95% EtOH) and 9 g of benzoic acid in solution with boiling deionized water were added as preservatives. After 10 min. of boiling, ~15 ml of diet was dispensed into 30-ml plastic cups and allowed to cool and dry in a laminar flow hood. Neonate larvae were surface sterilized for ~2 min. in a 0.25% bleach solution, rinsed with deionized water, and placed in cups with diet. The cups were covered with plastic lids (PC100 1 oz. cups and lids, Jet Plastica, Harrisburg, PA). Trays

containing the cups were held in a room at 25°C and 60-70% RH. Because of mortality associated with larval interactions in the cups, cups were opened at ~4-6 wk and individual larvae were transferred to fresh diet cups to complete development. Larvae pupated in the diet cups. Teneral adults were left for at least 3 d until sclerotization of the cuticle was complete. Adults were fed green beans, carrots, or citrus foliage according to availability and intended experimental design. Field-collected adults were introduced when available from the field to renew the colony and avoid adaptation to rearing conditions.

Because the objective of mass-rearing has been to provide insects for experimental purposes, progress has been empirical and output-oriented. We initiated a series of experiments to quantify the effect of environmental parameters on the developmental physiology of *D. abbreviatus*.

Trial 1.

Soil (Metromix 500, Scotts, Marysville, OH) was dried at 60°C for 4 d in an analytical oven and weighed. Water was added to dry soil to generate soil moisture levels of 20 to 80% in 5% increments. We placed 105- and 180-d-old larvae from the colony into cups containing soil without diet and kept them at room temperature (~25°C). Fifteen larvae (reps) were removed randomly from the colony without regard to their weight and assigned to each treatment. Ambient relative humidity in the rearing room where the cups containing the larvae were stored fluctuated around 70%. Because lids do not form a perfect seal, soil moisture content declined by as much as 25% over the course of the experiment (12 wk). Larvae were observed every 2 wk for pupation. Dates of pupation and adult emergence were recorded until the end of the trial (12 wk).

Trial 2.

A second trial was designed to maintain more uniform temperature and moisture conditions throughout the trial. Treatments were reduced to 7 (20-80% soil moisture content in 10% increments) and prepared as in Trial 1. We randomly selected 68- and 180-d-old larvae from the colony and placed them into cups containing soil without diet. The younger larvae (68 d) were selected to represent late instars close to completion of development. Older larvae (180 d) were selected because their pupation was delayed, possibly due to inadequate conditions in the colony. Each treatment (larval age × moisture) was sealed in a plastic bag to minimize moisture loss. Bags containing larvae and soil were kept in a dark incubator at 25 ± 1°C. Larvae were observed every 2 d for pupation. Dates of pupation and adult emergence were observed until the end of the trial (14 wk). Fresh and dry weights were determined at the end of the trial for 5 insects from each treatment. Insects were weighed upon removal from their cups, dried in an analytical oven at 60°C for 4 d and weighed again. Data were analyzed by analysis of variance and Tukey's Honestly Significant Differences (HSD) test (Abacus Concepts 1996).

Results and Discussion

Rearing.

Adults, larvae, and eggs of *D. abbreviatus* have been produced continuously since 1992 using the method described. Current peak production is approximately 400 adults/wk.

Trials 1 and 2.

Total pupation increased in Trial 1 with increasing soil moisture and reached a plateau at 60 - 65% for both age classes of weevils (Fig. 1). Ambient temperature and humidity were not controlled in this trial. We suspect that soil moisture content in the cups containing soil and larvae declined over the course of the experiment. In Trial 2, capped cups were kept in an incubator in sealed plastic bags at constant temperature, minimizing moisture loss. Optimal soil moisture for pupation of 68-d-old larvae ranged between 50 and 70% with a large increase in mortality at 80%. At 20% soil moisture content, mortality was also high and no pupation occurred (Fig. 2A). The time required for pupation did not vary by treatment ($\alpha = 0.05$, ANOVA) although sample size was small because few insects had pupated at the extreme treatments by the conclusion of the experiment at 90 d.

Mortality of older larvae (180 d) in Trial 2 was also high at 20 and 80% soil moisture content (Fig. 2B). Pupation was similar for treatments between 30 and 70%. Time to pupation was greater at 80% compared with the remaining treatments ($\alpha = 0.05$, Tukey's HSD test). Mean ± SEM time to pupation for the remaining treatments was 38.4 ± 1.9 d ($n = 54$).

Early experience with the colony indicated an apparent high degree of variability in time required for pupation, often exceeding one year (Beavers 1982). However, in Trial 2, of those 68-d-old larvae subjected to favorable moisture conditions (50-70%),

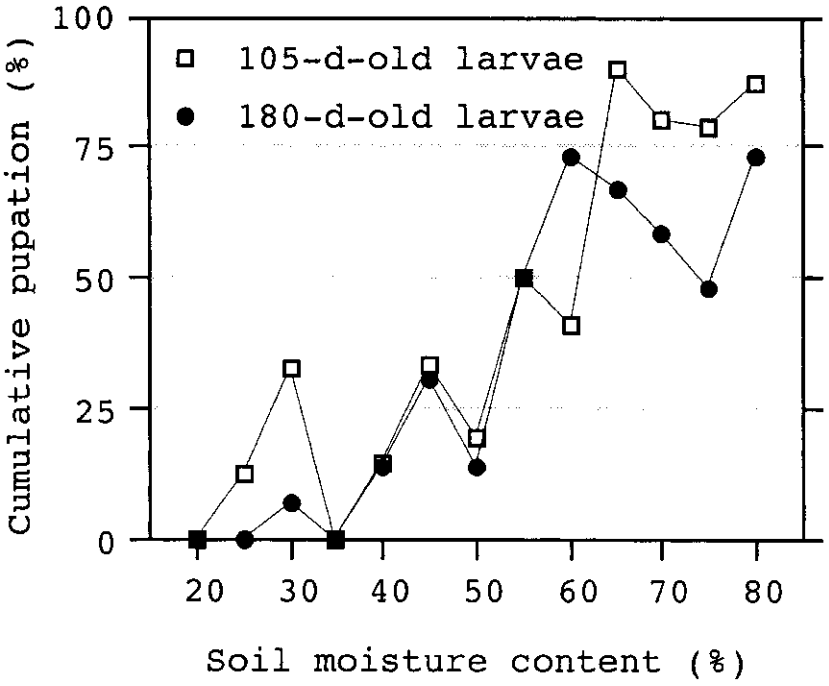

Fig. 1. Effect of initial soil moisture content on cumulative pupation of 105- and 180-d-old larval *D. Abbreviatus* after 12 wk.

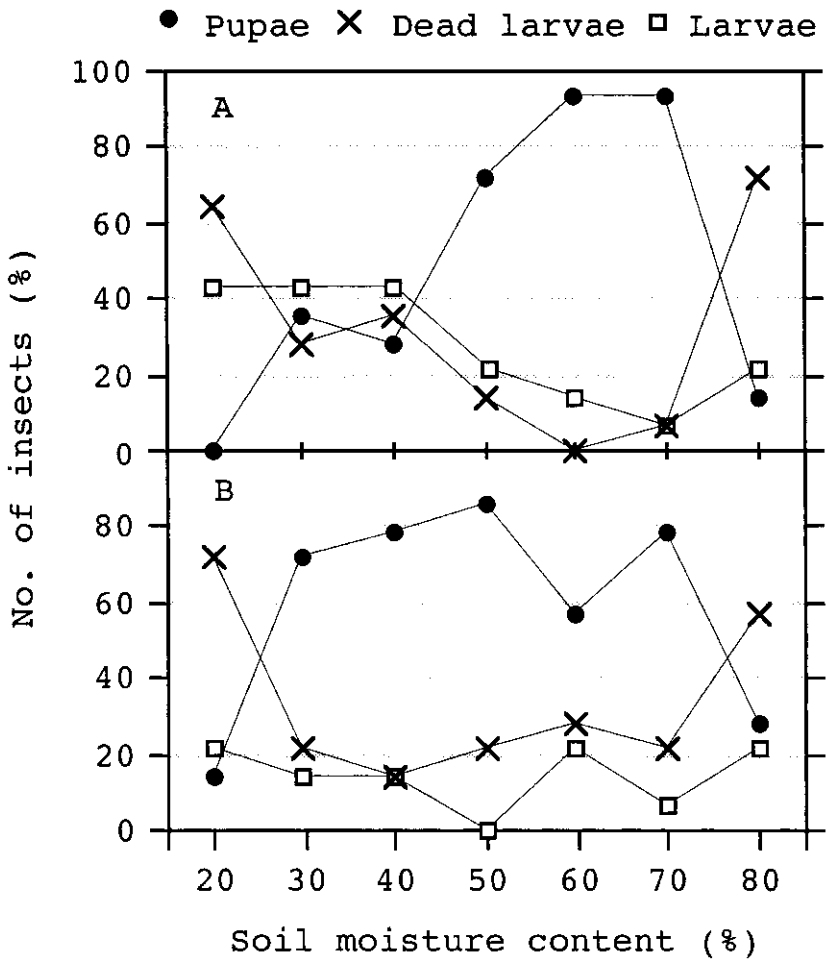

Fig. 2. Effect of constant soil moisture content on cumulative pupation and mortality of 68- (A) and 180-d-old (B) larval *D. abbreviatus*.

pupation occurred at 58 ± 3 d. The distribution of pupation times closely approached a normal distribution (Fig. 3). From these data, there does not appear to be genetic variability for rate of development.

There was no difference in the number of days to pupation of those 68-d-old larvae that pupated during Trial 2 (Table 1). The mean number of days (± SEM) to pupation of larvae transferred from diet to soil at 68 d was 58.2 ± 2.3 days after transfer (n = 47). Therefore, the mean development time of neonate larvae to pupation was 18 wk (~4.2 months). Fewer larvae survived at high (80%) and low (20-40%) soil moisture contents (Fig. 2A). Similarly, survival of 180-d-old larvae was low in the 20 and 80% treatments although in general, older larvae pupated over a greater range of moisture conditions compared with 68-d-old larvae (Fig. 2B). At 80% soil moisture content, the

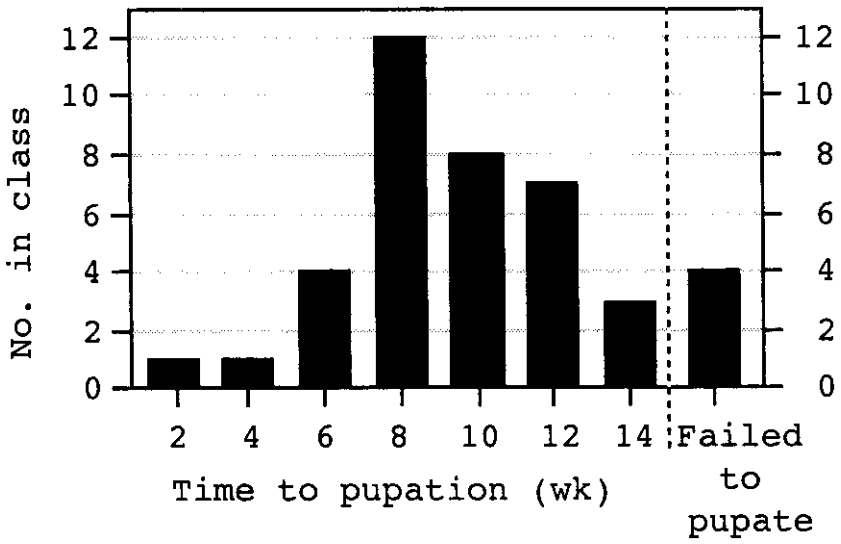

Fig. 3. Frequency distribution of time to pupation of 68-d-old larval *D. abbreviatus* in soil with moisture content of 50-70%.

development of 180-d-old larvae that pupated during the trial was significantly delayed compared with the remaining treatments (Table 1).

Soil moisture content did not affect the dry weights of insects at the end of Trial 2 (Pr > F = 0.12 and 0.14 for 68- and 180-d-old larvae, respectively). Final dry weights (mean ± SEM) were 122 ± 5 and 110 ± 6 mg for 68- and 180-d-old larvae, respectively. However, moisture content of insects at the end of the trial increased with increasing soil moisture content (Table 2). In the field, we expect that larvae of *D. abbreviatus* are capable of directed movements towards areas of preferred humidity in order to maintain their water content.

Barrow (1924) and Beavers & Selhime (1986) observed that heavy rainfall generally preceded emergence of adult Diaprepes in the field. Dry soil conditions may delay development resulting in an accumulation of dormant larvae or pupae in the soil. Subsequent rainfall may serve to synchronize completion of development, pupation, or adult emergence. Nothing is known about how larvae of *D. abbreviatus* move in the soil as a function of soil conditions (moisture content) and developmental stage. Wolcott (1934) observed that in arid and semi-arid regions of Puerto Rico, the weevil is present mostly in irrigated fields. It is possible that modern irrigation methods contribute to larval feeding damage by creating optimal conditions of soil moisture near the structural roots of citrus where the most severe feeding damage occurs.

Wolcott (1934) estimated the normal life cycle of *D. abbreviatus* to be approximately 1 year in the field based on observations of a small number of weevils reared in his laboratory in Puerto Rico. Beavers (1982) reported development times of slightly longer than 1 year for both sexes when reared on artificial diet. However, no attempt was made to control or monitor moisture conditions of the artificial diet. Our data suggest that Beavers' estimate of development time was largely an artifact of the rearing conditions, specifically, the progressive drying of the diet over time under laboratory conditions. Given optimal soil moisture (60 ± 10% by weight) and temperature condi-

TABLE 1. TIME REQUIRED FOR PUPATION AND EMERGENCE OF ADULTS AFTER TRANSFER OF 2 AGE CLASSES OF LARVAE OF *DIAPREPES ABBREVIATUS* TO SOIL WITH DIFFERENT WATER CONTENTS.

	68-d-old larvae						180-d-old larvae					
	Days to pupation			Teneral period (d)			Days to pupation			Teneral period (d)		
% water	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*
20	—		0	—		0	40.0 a	0.0	2	—		0
30	50.0 a	2.8	5	21.3 a	1.4	3	41.2 a	3.3	10	22.3 a	0.9	7
40	62.5 a	3.9	4	22.0 a	0.0	1	34.2 a	3.1	11	21.4 a	0.5	7
50	59.0 a	5.0	10	19.1 a	0.7	7	39.3 a	3.4	8	20.3 a	0.7	8
60	55.1 a	5.2	13	20.0 a	0.4	10	36.3 a	3.4	8	20.3 a	0.7	8
70	61.1 a	4.5	13	20.2 a	0.7	9	40.5 a	5.6	11	19.0 a	0.5	10
80	67.0 a	0.7	2	18.0 a	1.4	2	68.0 b	7.6	4	26.0 a	4.2	2

Means within columns followed by the same letter do not differ ($\alpha = 0.05$, ANOVA and Tukey's HSD).

TABLE 2. WATER CONTENT OF 2 AGE CLASSES OF *DIAPREPES ABBREVIATUS* AFTER 90 D ON SOIL WITH DIFFERENT SOIL MOISTURE CONTENT.

Soil water content (%)	Insect water content (%) (Mean ± SEM, n = 5)	
	68-d-old larvae	180-d-old larvae
20	44 ± 2 a	33 ± 8 a
30	53 ± 2 ab	33 ± 9 a
40	56 ± 2 b	43 ± 4 ab
50	60 ± 2 bc	60 ± 0 bc
60	67 ± 2 cd	67 ± 3 c
70	68 ± 2 cd	67 ± 2 c
80	72 ± 3 d	70 ± 2 c

Means within columns followed by the same letter do not differ (α = 0.05, Tukey's HSD).

tions (as yet to be determined), our estimate of the development time of *D. abbreviatus* reared on artificial diet and transferred to soil is approximately 18 wk at 25°C. It must be noted that we used larvae at moderate to advanced stages of growth (68 and 180 d) from the colony where moisture content of the diet and temperature were not controlled. Development time from neonate to adult may be even less if neonates are subjected to optimal conditions of moisture and temperature beginning at egg eclosion, and are provided with diet throughout the larval period. We are currently testing combinations of moisture and temperature to identify optimal rearing conditions.

ACKNOWLEDGMENTS

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PROCRYPTOTERMES EDWARDSI, A NEW DRYWOOD TERMITE (ISOPTERA: KALOTERMITIDAE) FROM JAMAICA

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ABSTRACT

Procryptotermes edwardsi n. sp. is described from soldiers and imagos collected in Jamaica. It is the second species of New World *Procryptotermes* and the smallest species of *Procryptotermes* known worldwide.

Key Words: taxonomy, new species, Neotropics, West Indies, Caribbean

RESUMEN

Se describe *Procryptotermes edwardsi* n. sp. de soldados e imagos colectados en Jamaica. Es la segunda especie de *Procryptotermes* en el nuevo mundo y la especie más pequeña de este genero mundialmente.

Procryptotermes Holmgren is a coastal and island genus of drywood termite that consists of 11 species recorded from southern India, Australia, islands of the Indian

Ocean, Polynesia, and the West Indies (Krishna 1961, 1962; Roonwal and Chhotani 1963, Gay 1975, Thakur 1975, Bose 1979). The soldier of *Procryptotermes* is distinguished from other kalotermitid genera by long, sickle-shaped mandibles and weak to moderate head capsule phragmosis. The imago of *Procryptotermes* is similar to that of *Cryptotermes* Banks in which the median vein is unsclerotized and intersects the radial sector near mid-wing.

Until now, *P. corniceps* (Snyder) (Snyder 1923) was the only *Procryptotermes* species known from the New World (Constantino 1998). During a 1997 termite expedition to Jamaica, a new species of *Procryptotermes* was collected. The descriptions of the soldier and imago of *Procryptotermes edwardsi* n. sp are provided herein.

Materials and Methods

Morphometrics of specimens preserved in 85:15 ethanol:water were made with a stereomicroscope fitted with a calibrated ocular micrometer. General measurements are as described in Roonwal (1970). In soldiers, head length to genal horns is measured from the median posterior of head to tip of horns, and frontal flange width is equal to head width at the frontal flange. Scanning electron micrographic prints were scanned at 600 dpi, the digital image outline traced using photograph-enhancing software (Photo Magic, Micrografx, Inc., Richardson, TX), the background converted to black, and scale bar digitally redrawn.

Latitude and longitude coordinates were measured at collection sites using a Magellan GPS 2000 hand-held global positioning receiver (Magellen Systems Corp, San Dimas, CA). Coordinates of collection sites were converted to decimal degrees and mapped (Fig. 2) using ArcView GIS version 3.0a software and relevant map data from Digital Map of the World version 1.0 (Environmental Systems Research Institute, Inc. Redlands, CA).

The holotype soldier and morphotype imago will be deposited in the collection of the American Museum of Natural History, New York. Paratype soldiers and imagos will be deposited in the National Museum of Natural History (Smithsonian Institution), Washington, DC; the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville; and in the authors' collection at the University of Florida Research and Education Center, Ft. Lauderdale.

Procryptotermes edwardsi Scheffrahn, **New Species**

Imago (Table 1).

General color yellow-brown except as noted. Head darker brown on vertex between ocelli; epicranial suture partly delineated by lighter patches. Pronotum paler toward middle; T-shaped pattern on midline even paler. Femora and anteclypeus pale yellow. Wing scales yellow-brown; sclerotized veins, including costa, subcosta, radius, and radial sector brown; wing membranes with very faint yellow-brown tint. Head capsule with few scattered short or very short bristles; lateral margins of pronotum fringed with more numerous alternating patterns of short and long bristles. Short and long bristle pattern also on tergites and sternites. Antennae with 13-16 articles, usually 15 or 16; relative length formulae variable, usually 2 > 3 > 4 = 5, 2 = 3 = 4 = 5, or 2 > 3 = 4 = 5. Ocellus white, oval, touching eyes; eyes not noticeably large or small, slightly triangulate with straight margins bordering antennae, ocelli, and posteroventral margins of head. Pronotum about as wide as head capsule; anterior margin broadly

TABLE 1. MEASUREMENTS OF *PROCRYPTOTERMES EDWARDSI* IMAGO.

Measurement in mm (n = 3♀, 9♂ from 3 colonies)	Range	Mean ± S. D.	Morphotype
Head length with labrum	1.05-1.21	1.12 ± 0.052	1.13
Head length to postclypeus	0.92-1.01	0.97 ± 0.032	1.01
Head width, maximum at eyes	0.83-0.88	0.85 ± 0.017	0.87
Eye diameter, maximum	0.25-0.29	0.26 ± 0.013	0.28
Eye to head base, minimum	0.11-0.14	0.12 ± 0.012	0.14
Ocellus diameter, maximum	0.11-0.13	0.12 ± 0.006	0.12
Pronotum, maximum length	0.59-0.67	0.62 ± 0.024	0.67
Pronotum, maximum width	0.77-0.85	0.81 ± 0.030	0.85
Total length with wings	7.31-8.38	7.88 ± 0.37	7.74
Total length without wings	2.98-5.33	4.15 ± 0.80	4.05
Forewing length from suture	5.68-6.25	6.02 ± 0.15	6.11
Forewing, maximum width	1.42-1.65	1.54 ± 0.086	1.60

concave, posterior margin straight or weakly incised; posterolateral corners broadly rounded. Median veins unsclerotized, joining radial sectors at about 3/5 wing length from sutures; radial sectors each with 4-5 anterior branches. Arolia present.

Soldier (Fig. 1A-C, Table 2).

Head orange-brown to dark orange-brown at postclypeus, grading unevenly to pale yellow near occiput; epicranial ("Y") suture delineated by faint, narrow, and even lines. Mandibles dark red-brown distally, grading to light orange-brown at basal 1/5-1/3. Anteclypeus hyaline; labrum orange-yellow; antennae orange-brown. Pronotum pale yellow-brown with hyaline midline. Scattered medium and short bristles on head capsule and pronotum. Frontal flange very slightly elevated and marked by very weak striated rugosity. Flange divided by flat median plane or weak concavity; plane or concavity continuous with vertex. Slope of frons plane angled about 35-40º below plane of vertex. Genal horns small, triangulate; axes diverging laterally in dorsal view. Frontal protuberances absent. Labrum spatulate or forming apical point when anterolateral corners curved. Eye spots hyaline, elliptical; posterior to and even with antennal fossae. Mandibles very long and curving evenly about 70º in distal 2/5. Left mandible with three marginal teeth; first and second narrow and conical, third weak and broad-based. Right mandible with two acute, shelflike marginal teeth. Antennae with 12-14 articles; relative length formulae variable; usually $2 > 3 < 4 = 5$, $2 > 3 = 4 = 5$, or $2 = 3 > 4 = 5$. Pronotum narrower than head; anterior margin weakly incised, posterior margin rounded or with slight concavity near middle.

Comparisons.

The imago and soldier castes of *P. edwardsi* are the smallest *Procryptotermes* described. The imago of *P. edwardsi* is very close to *P. corniceps* except that the former is smaller in all measurements taken (without overlap) and has about two fewer antennal articles.

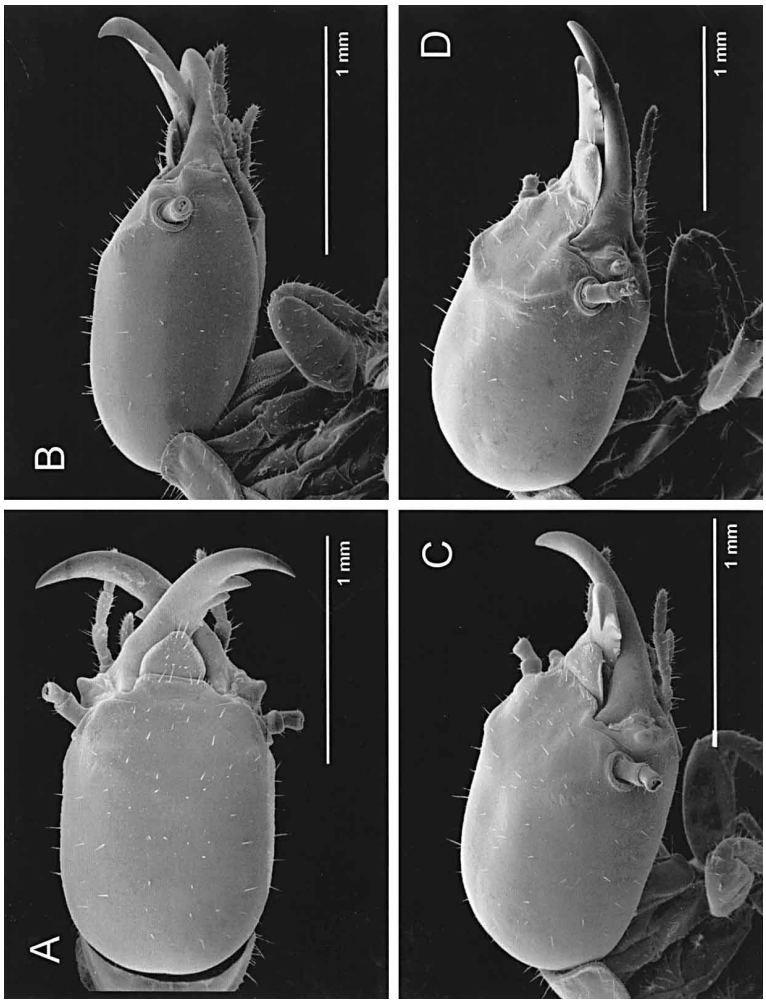

Fig. 1. Dorsal (A), lateral (B), and oblique (C) views of soldier head capsule of *Procryptotermes edwardsi* from Cousin Cove, Jamaica. Oblique view of *P. corniceps* soldier (D) from Baños de Coamo, Puerto Rico. Antennae partially removed for clarity.

TABLE 2. MEASUREMENTS OF *PROCRYPTOTERMES EDWARDSI* SOLDIER.

Measurement in mm (n = 13 from 3 colonies)	Range	Mean ± S. D.	Holotype
Head length to tip of mandibles	2.10-2.35	2.21 ± 0.087	2.10
Head length to median flange	0.86-1.04	0.95 ± 0.047	0.94
Head length to genal horns	1.26-1.36	1.32 ± 0.028	1.31
Frontal flange width	0.82-0.87	0.83 ± 0.015	0.85
Genal horns outside width	0.91-0.96	0.94 ± 0.019	0.91
Head width, maximum	1.00-1.05	1.02 ± 0.013	1.01
Head height, excluding postmentum	0.75-0.83	0.80 ± 0.024	0.78
Pronotum, maximum width	0.84-0.93	0.89 ± 0.029	0.87
Pronotum, maximum length	0.52-0.64	0.57 ± 0.028	0.56
Left mandible length; tip to ventral condyle	1.11-1.23	1.18 ± 0.031	1.19
Total length	3.47-5.10	4.25 ± 0.48	4.16

The soldier of *P. edwardsi* is close to *P. corniceps*, but the former is smaller in all measurements. The frontal flange of the *P. corniceps* soldier is acutely elevated to form a continuous ridge or brow between the vertex and frons (Fig. 1D). The frontal flange of *P. edwardsi* is only faintly elevated laterally without forming a distinct or continuous ridge between the vertex and frons (Fig. 1B, 1C).

Type Material.

Holotype soldier and 4 paratype soldiers: Jamaica, Cousin Cove,18.441ºN 78.235ºW, 31.v.1997 (coll. no. JA769). Morphotype imago, 5 paratype soldiers and 5 paratype imagos: Jamaica, Discovery Bay, 18.479ºN 77.440ºW, 24.v.1997 (JA095). Three paratype imagos: same data as JA095, second colony from location (JA097). Three paratype imagos: Jamaica, 2.3 km N Frenchman, 17.851ºN 77.491ºW, 29.v.1997 (JA535). Three paratype soldiers: Jamaica, Font Hill, 18.047ºN 77.947ºW, 30.v.1997 (JA641). Additional *P. edwardsi* material examined: Jamaica, Hellshire Point, 17.882ºN 76.909ºW; imagos, soldiers, pseudergates; 28.v.1997 (JA433). All above material taken collectively by P. Ban, J. A. Chase, J. Krecek, B. Maharajh, J. R. Mangold, and Y. Roisin.

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Etymology.

Named after Jeff Edwards, Dead Bug Edwards Termite Company, Plantation, Fla., for his financial support and volunteerism benefiting the study of drywood termite biology and control.

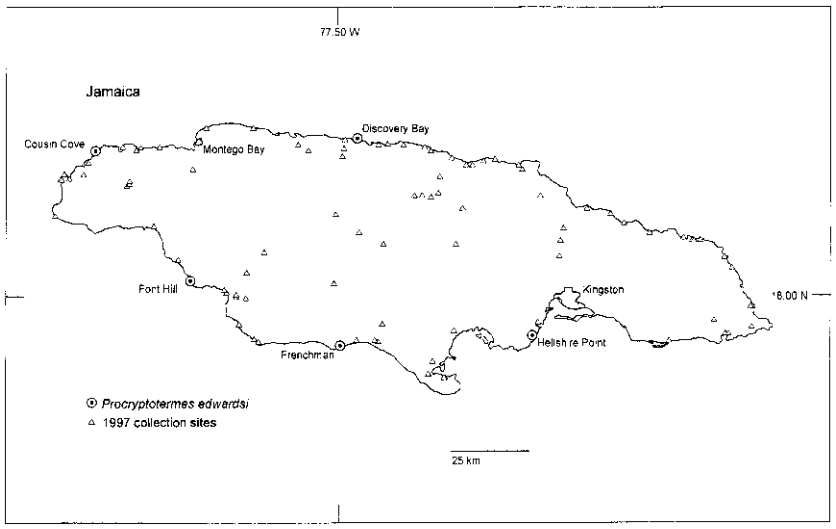

Fig. 2. Localities of *Procryptotermes edwardsi* and 1997 termite collection sites for Jamaica, West Indies.

BIOLOGY

In May 1997, we collected colonies of *P. edwardsi* from 5 of 85 sites surveyed for termites in Jamaica (Fig. 2). Of these 85 sites, 78 yielded one or more kalotermitid samples in the genera *Cryptotermes*, *Glyptotermes*, *Incisitermes*, *Neotermes*, and/or *Procryptotermes*. Colonies of *P. edwardsi* were encountered in dead limbs of various woody hosts in the type localities, all of which were coastal habitats broadly encompassing Jamaica. Ten of 13 colonies contained winged imagos, suggesting that dispersal flights commence in late spring and early summer.

ACKNOWLEDGMENTS

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THE CITRUS LEAFMINER *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE)IN SOUTH TEXAS: INCIDENCE AND PARASITISM

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ABSTRACT

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was first reported in the Lower Rio Grande Valley of Texas in August, 1994. We surveyed about 40 orchards in 1995 and 20 in 1996. Percentage of leaf infestation by the leafminer was lowest on the spring flush, and increased significantly in the early summer (May-July) and late summer flushes (Aug.-Oct.) through to late fall (Nov.-Dec.). Numbers of citrus leafminer immatures usually ranged from 0-6.8 per leaf. Several native parasite species were identified from the surveys, including 9 species of parasites from 3 families, Eulophidae, Proctotrupidae and Ceraphronidae. The most abundant native parasitoid was *Zagrammosoma multilineatum* (Ashmead) (Eulophidae). Less dominant parasitoids were the eulophids *Horismenus* sp., *Closterocerus* sp., *Neochrysocharis* sp., *Pnigalio* sp., and *Tetrastichus* sp. Percentage parasitism by native parasitoids usually ranged from 5-10%. The exotic parasitoid *Ageniaspis citricola* Logvinoskaya (Encyrtidae) was released in February-April 1995 and August-October 1996.

Key Words: biological control, parasites, population dynamics

RESUMEN

El minador de los cítricos, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), fue reportado por primera vez en la región llamada "Lower Rio Grande Valley" de Texas en agosto de 1994. Inspeccionamos aproximadamente 40 huertas en 1995 y 20 en 1996. El porcentaje de hojas infestadas por el minador tuvo su nivel más bajo durante el crecimiento del follaje de la primavera y aumentó significativamente a principios de verano (mayo-julio) y a finales de verano (agosto-octubre) hasta finales de otoño (nov.-dec.). El número de larvas del minador generalmente varió de 0 a 6.8 por hoja. Se identificaron varias especies nativas de parásitos durante estas inspecciones, incluyendo a 9 especies en 3 familias, Eulophidae, Proctotrupidae y Ceraphronidae. La especie de parasitoide nativa más abundante fue *Zagrammosoma multilineatum* (Ashmead) (Eulophidae). Entre los parasitoides menos dominantes estuvieron los Eulophidae *Horismenus* sp., *Closterocerus* sp., *Neochrysocharis* sp., *Pnigalio* sp., y *Tetrastichus* sp. El porcentaje de parasitismo por parasitoides nativos generalmente varió del 5 al 10%. El parasitoide exótico *Ageniaspis citricola* Logvinoskaya (Encyrtidae) fue liberado en los meses de febrero y abril de 1995 y en agosto y octubre de 1996.

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a serious pest of citrus and related plant species throughout Southern Asia, Australia and east Africa, and is native to eastern and southern Asia (Beattie 1993). In May 1993, citrus leafminer was discovered to have invaded southern Florida with over 90% infestation reported in Dade County (Heppner 1993a, see also Hoy 1996). By late 1994, the invasion had spread beyond Florida to Louisiana and Texas. The leafminer begins to damage the host plant as soon as its eggs hatch. The larvae bore through the leaf epidermis, ingesting the sap and producing a chlorotic leaf patch. Citrus leafminer may prevent young leaves from expanding, causing them to remain curled and twisted. Citrus leafminer may also attack succulent stems and fruits. After the miner has finished feeding, other insects such as the aphids; e.g., *Aphis gossypii* Glover and mealybugs, *Planococcus citri* (Risso) may continue feeding on the damaged area. Secondary effects of citrus leafminer damage may also include leaf desiccation or invasion by fungi and bacteria (Achor et al. 1997). Spatial distribution of eggs and larvae have been found statistically aggregated within the canopy. Egg and larval densities are also significantly higher on apical leaves on young branches compared with older, middle and bottom leaves on young branches of lime (Peña & Schaffer 1997).

Citrus leafminer was first reported in the Lower Rio Grande Valley of Texas in August 1994 (French et al. 1994). Surveys conducted immediately following the discovery revealed that the pest was already well established in several orchards and in a large citrus nursery. Practically all Texas citrus is currently grown in the Lower Rio Grande Valley, with about 35,000 acres estimated at an economic value of $130-150 million. The leafminer invasion could constitute a serious setback to an industry still recovering from devastating freezes in 1983 and 1989 during which acreage declined from ≈70,000 to 12,000. Acreage then increased to about 35,000 acres. Furthermore, citrus leafminer could spread throughout the southeast United States, probably as far north as Georgia.

Biological control may be a useful tool in suppressing populations of this insect (Hoy & Nguyen 1994a). Effective chemical control is difficult because this pest can develop resistance to pesticides, and its larvae are protected from insecticides by the leaf cuticle. The pupae are protected by the rolled leaf margins. A number of natural ene-

mies have been found for citrus leafminer, including 39 species of parasites from 7 families, mostly Chalcidoidea (Heppner 1993b). Ten species of chalcidoid parasites were reared from the citrus leafminer in Thailand (Hoy & Nguyen 1994a), with *Ageniaspis citricola* Logvinoskaya probably being the most significant. Schauff (1998) described two new species of Eulophidae reared from the citrus leafminer from Puerto Rico and Colombia. Currently, nearly 80 species of parasitoids have been reared from citrus leafminer throughout the world (LaSalle & Peña 1997, citing Schauff et al. [submitted]). Many are indigenous parasitoid species that have parasitized citrus leaf miner as it has spread from the tropics—over 20 such species are listed by LaSalle & Peña (1997, citing Schauff et al. [submitted]).

In February-April, 1995, and August-October, 1996, the parasitoid *Ageniaspis citricola* Logvinoskaya (Encyrtidae) was released in the Lower Rio Grande Valley in collaboration with J. Goolsby (APHIS Mission Biological Control Center, TX). The objective of this study is to survey the citrus leafminer incidence and native complex parasitism, and determine the degree of establishment of *A. citricola.*

Materials and Methods

Survey for citrus leafminer and native parasites.

We surveyed about 40 commercial orchards in the Lower Rio Grande Valley in 1995 and 20 in 1996, over a survey area of ≈100 mi. in diameter (Fig. 1). No samples

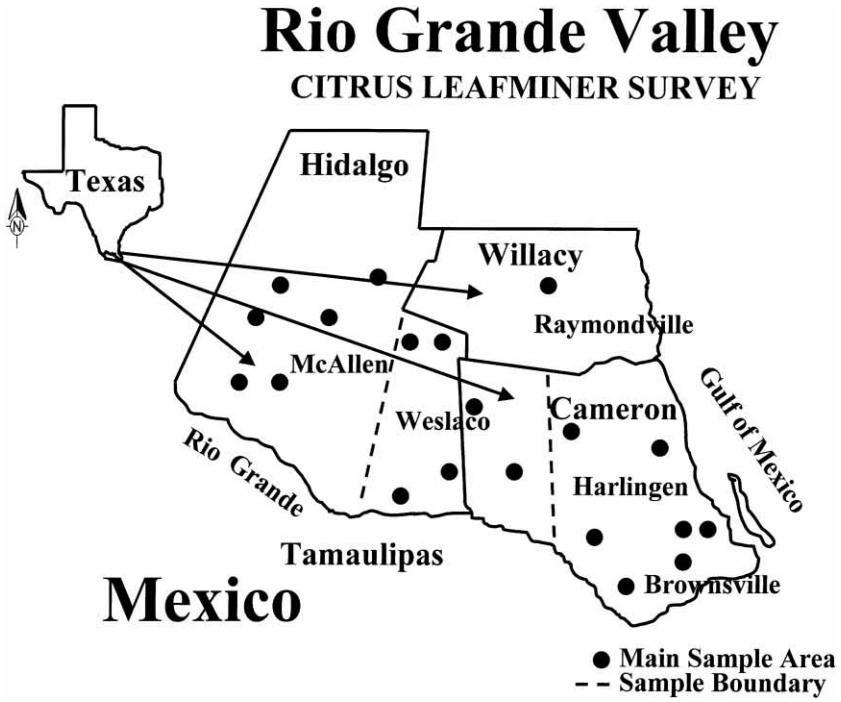

Fig. 1. Sample locations in the Lower Rio Grande Valley (1995-1996).

were collected in January of both years due to the cold conditions (below freezing temperatures at certain periods) in the area. Six-26 leaf terminals were randomly collected per site to determine leaf infestation from February to December, 1995. In 1996, twelve leaf terminals were collected from 1 orchard from the east, central and western regions of the valley every 3 weeks. Leaf terminals were also collected from another 3-5 commercial orchards across the valley that were sampled randomly every 1-2 months. Percentage parasitism by the endemic parasite complex was calculated from a collection of 25-100 leaves per site at various times throughout 1995-1996. Parasitized citrus leafminer were reared through in the laboratory for species identification and to determine seasonal distribution of their native and exotic parasites. Parasites reared in the laboratory were identified by Dr. Michael E. Schauff (USDA Systematics Laboratory, Beltsville, MD) and Dr. James B. Woolley (Department of Entomology, Texas A&M University, College Station, TX). Voucher specimens were kept in the insect museums of the latter institutes.

Release and evaluation of an exotic parasite.

At several sites in the 3 regions of the Lower Rio Grande Valley, the exotic parasitoid *Ageniaspis citricola* was released (obtained from M. A. Hoy, University of Florida and R. Nguyen, Division of Plant Industry, FL) in February-April, 1995 and August-October 1996. Orchards that had a substantial population of first instar citrus leafminer on very young flushes were selected as release sites. In 1995, a total of 868 and 85 adult female parasites were released in the central (February, April, July) and western (July) parts of the valley. In 1996, 626 (August) and 480 (October) adult female parasites were released in the eastern and central regions, respectively. Subse-

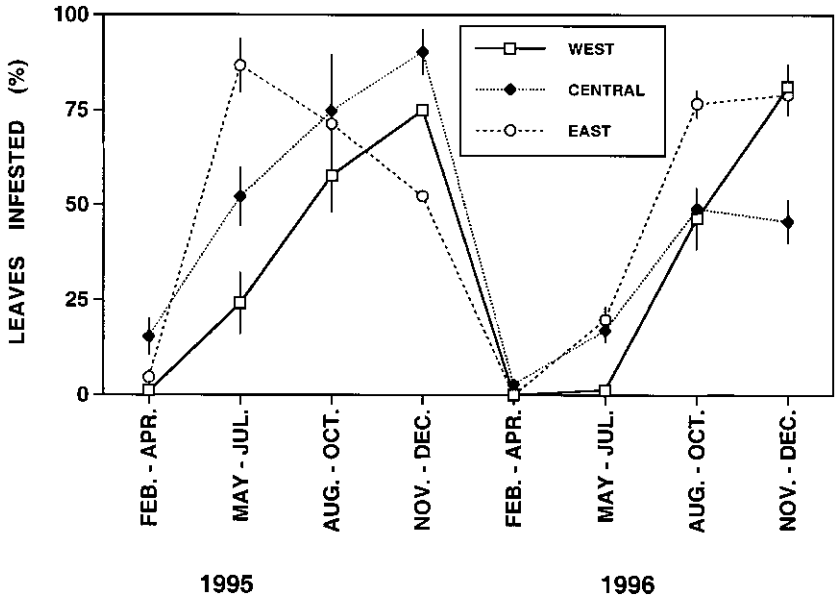

Fig. 2. Mean percentage leaves infested (± SEM) by citrus leafminer in 3 regions of the Lower Rio Grande Valley (1995-1996).

quent evaluation involved a random collection of up to 140 older leaves per site at various times after release. The number of *A. citricola* pupae and adults that emerged were counted and recorded.

Results and Discussion

Survey for citrus leafminer and native parasites.

In 1995, percentage leaf infestation was lowest on the spring flush, and increased significantly on the early summer (May-July) and late summer (Aug.-Oct.) flushes through to late fall (Nov.-Dec.) (Fig. 2). Mean percentage leaf infestation (average across the 3 regions) increased steadily throughout 1995 until the winter: 7.03% (Feb.-Apr.), 54.3% (May-Jul.), 67.6% (Aug.-Oct.), and 72.5% (Nov.-Dec.). Mean percentage infestation (across the 3 regions) began to increase by the following spring: 12.8% (May-Jul.), 57.3% (Aug.-Oct.), and 68.6% (Nov.-Dec.). The total numbers of leaf-

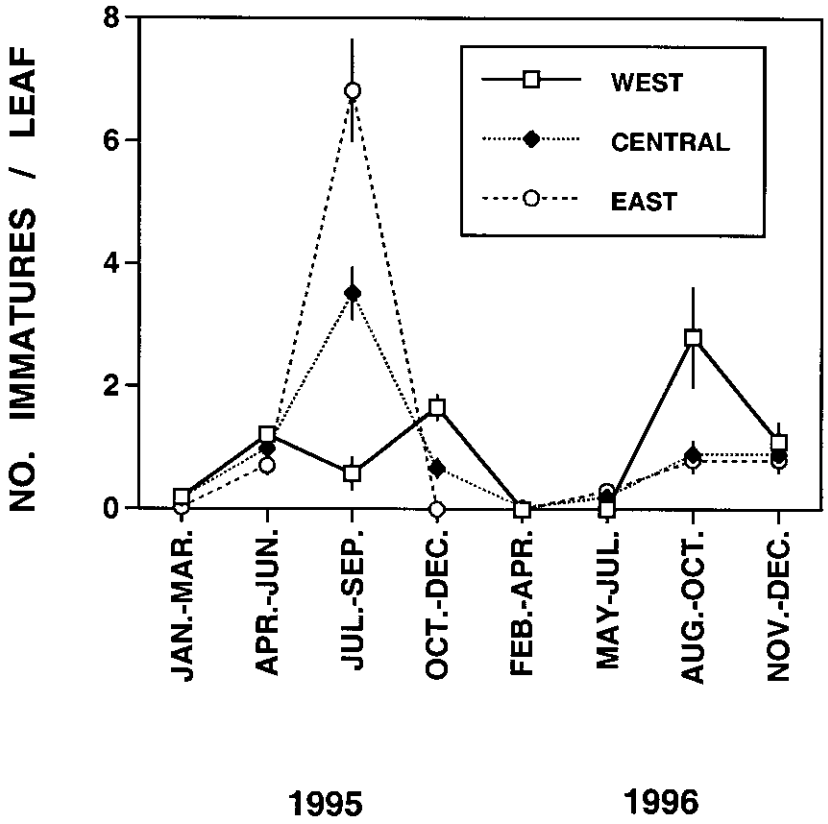

Fig. 3. Mean number of immature citrus leafminer per leaf (± SEM) in 3 regions of the Lower Rio Grande Valley (1995-1996).

TABLE 1. MEAN NUMBERS OF CITRUS LEAFMINER AT DIFFERENT STAGES PER LEAF (± S. E.) IN THREE REGIONS OF THE LOWER RIO GRANDE VALLEY OF TEXAS IN 1995 AND 1996.

	West			Central			East		
Date	Eggs	Larvae	Pupae	Eggs	Larvae	Pupae	Eggs	Larvae	Pupae
Jan-Mar '95	0.048 (0.022)	0.145 (0.036)	0.000 (0.000)	0.003 (0.003)	0.174 (0.024)	0.000 (0.000)	0.000 (0.000)	0.016 (0.012)	0.008 (0.008)
Apr-Jun '95	0.348 (0.050)	0.822 (0.080)	0.040 (0.012)	0.195 (0.034)	0.735 (0.071)	0.056 (0.022)	0.026 (0.026)	0.684 (0.137)	0.000 (0.000)
Jul-Sep '95	0.053 (0.053)	0.526 (0.246)	0.000 (0.000)	0.957 (0.215)	2.429 (0.353)	0.121 (0.036)	3.389 (0.585)	3.426 (0.404)	0.000 (0.000)
Oct-Dec '95	0.452 (0.118)	0.839 (0.123)	(0.366) (0.088)	0.061 (0.030)	0.576 (0.138)	0.030 (0.021)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Feb-Apr '96	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.024 (0.013)	0.002 (0.002)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
May-Jul '96	0.000 (0.000)	0.007 (0.003)	0.001 (0.001)	0.034 (0.014)	0.123 (0.038)	0.022 (0.009)	0.107 (0.037)	0.187 (0.052)	0.030 (0.008)
Aug-Oct '96	0.854 (0.276)	1.881 (0.711)	0.093 (0.023)	0.055 (0.021)	0.691 (0.208)	0.119 (0.052)	0.152 (0.062)	0.522 (0.139)	0.094 (0.022)
Nov-Dec '96	0.022 (0.019)	0.798 (0.305)	0.223 (0.069)	0.310 (0.145)	0.553 (0.154)	0.029 (0.020)	0.115 (0.065)	0.509 (0.172)	0.210 (0.071)

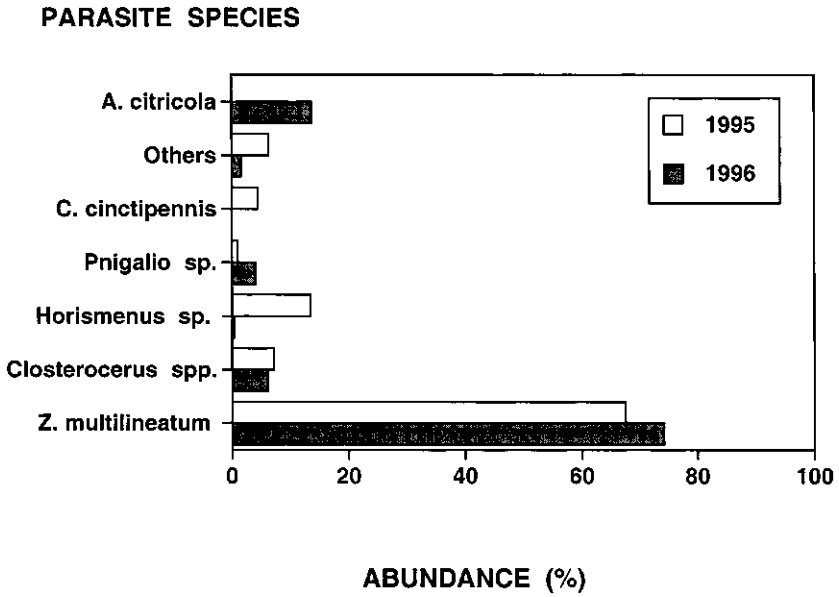

Fig. 4. Overall percentage abundance of the native *Zagrammosoma multilineatum, Closterocerus* sp., *Horismenus* sp., *Pnigalio* sp., *Closterocerus cinctipennis* and the exotic *(Ageniaspis citricola)* parasitoids of the citrus leafminer. "Others" include the natives *Neochrysocharis* sp., *Tetrastichus* sp., Proctotrupidae (1995), and Ceraphronidae (1996).

miner immatures ranged from 0-6.8 per leaf, with peak samples collected in July to September, 1995 from the East region (mean immatures ≈6.8) and in August to October, 1996 from the West (mean immatures ≈2.8) (Fig. 3). Table 1 presents the mean numbers of citrus leafminer at different stages per leaf in the three regions of the Lower Rio Grande Valley in 1995 and 1996 (see Table 1). Similar trends were reported by Peña et al. (1996) for Florida, wherein leafminer densities were found to increase from spring through fall, and declined during the winter.

Several native parasite species were identified from the surveys, including 9 species of parasites from 3 families, Proctotrupidae, Ceraphronidae and the more common Eulophidae (Fig. 4). The most abundant native parasitoid was *Zagrammosoma multilineatum* (Ashmead), which comprised 68% and 74% of the parasitoid complex sampled in 1995 and 1996, respectively. Less dominant parasitoids were the eulophids *Horismenus* sp., *Closterocerus* sp., *Neochrysocharis* sp., *Pnigalio* sp., *Tetrastichus* sp. and a few others that were unidentified. In 1996, *Ageniaspis citricola* was collected at the site of release and this exotic species was found to be a substantial percentage (17%) of all the parasites recovered (see Fig. 4). Percentage parasitism by native parasitoids usually ranged from ≈5-10%, but showed extremes in the samples taken from the east and central region, where parasitism ranged from 0-24.4% and 0-34.9%, respectively (Fig. 5). Seasonal distribution of the native parasites and the exotic species, *A. citricola*, are shown in Figs. 6A and 6B. In 1995 and 1996, the parasites were mostly abundant from August through December. Following the releases of *A. citricola* in August and September 1996, peak numbers of *A. citricola* were about as

high as those recorded for the native parasite, *Z. multilineatum,* in late August 1996. As may be expected, parasitization patterns of citrus leafminer during the two years (Fig. 5) were broadly similar to percentage leaf infestation (Fig. 2). The native parasitoid complex of south Texas was similar to those at Colima (Perales-Gutiérrez et al. 1996a and 1996b), and perhaps to a lesser extent, to Tamaulipas, in Mexico (Martínez-Bernal & Ruíz-Cancino 1996). In contrast, the most dominant native parasitoid of citrus leafminer in Florida was the eulophid *Pnigalio minio* (Walker) which comprised ≈80% of parasitoids which emerged from parasitized miners (Peña et al. 1996). Only 3 individual *Pnigalio* sp. was reared from our samples.

Release and evaluation of an exotic parasite.

The release of the exotic parasitoid *Ageniaspis citricola* in Feb.-Apr. 1995 was not successful because subsequent evaluations revealed that no parasitoids were recovered at the sites. The unsuccessful recoveries may be due to weather conditions and availability of suitable hosts in the valley at the time of release. Mean temperature, cumulative rainfall and relative humidity were: 18.9°C (66.1°F), 0.4 cm (0.16 in),

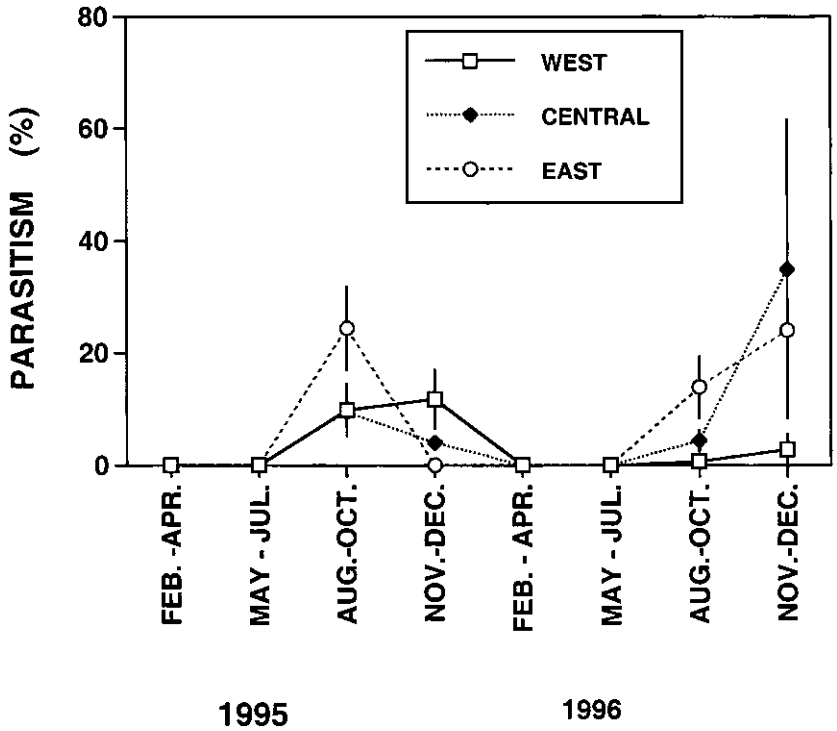

Fig. 5. Mean percentage parasitism (± SEM) by native parasites of citrus leafminer in 3 regions of the Lower Rio Grande Valley (1995-1996). Percentage parasitism by native parasitoids usually ranged from 5-10%, but showed extremes in the samples taken from the east and central region, where parasitism ranged from 0-24.4% and 0-34.9%, respectively.

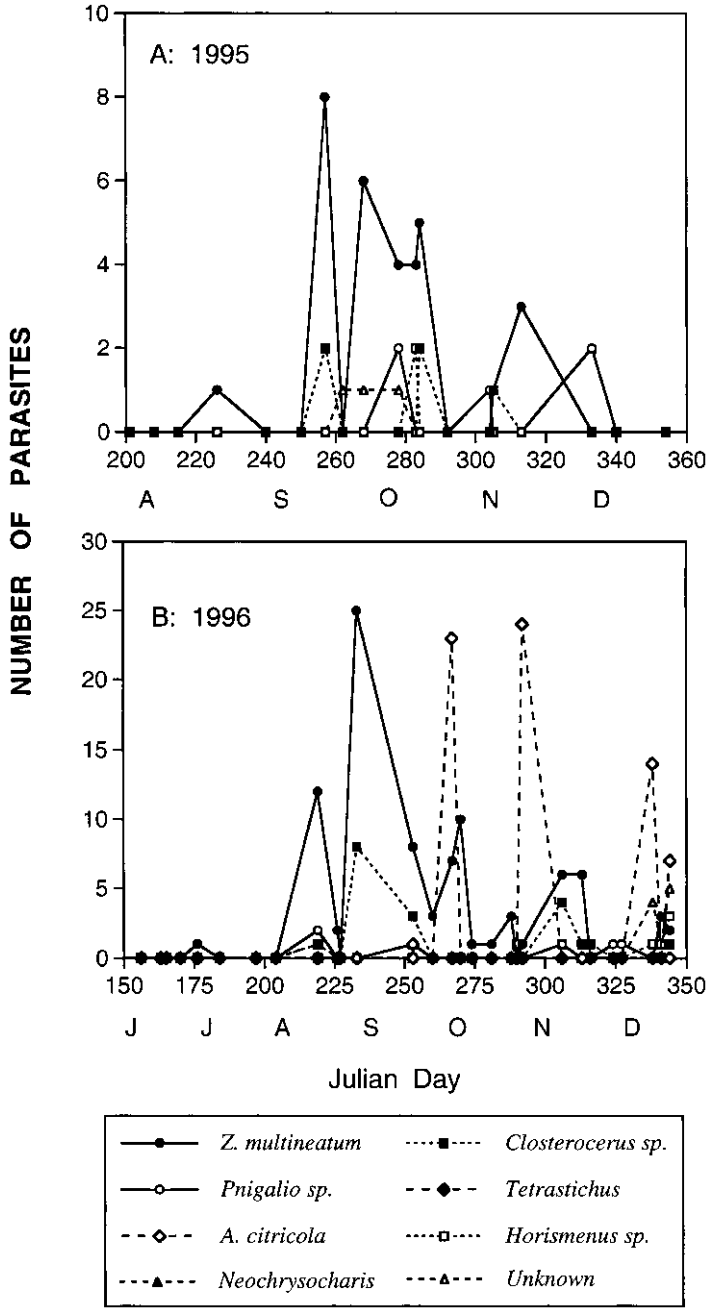

Fig. 6. Total number of parasites of citrus leafminer reared in the laboratory from leaf samples collected in the Lower Rio Grande Valley in 1995 (A) and 1996 (B).

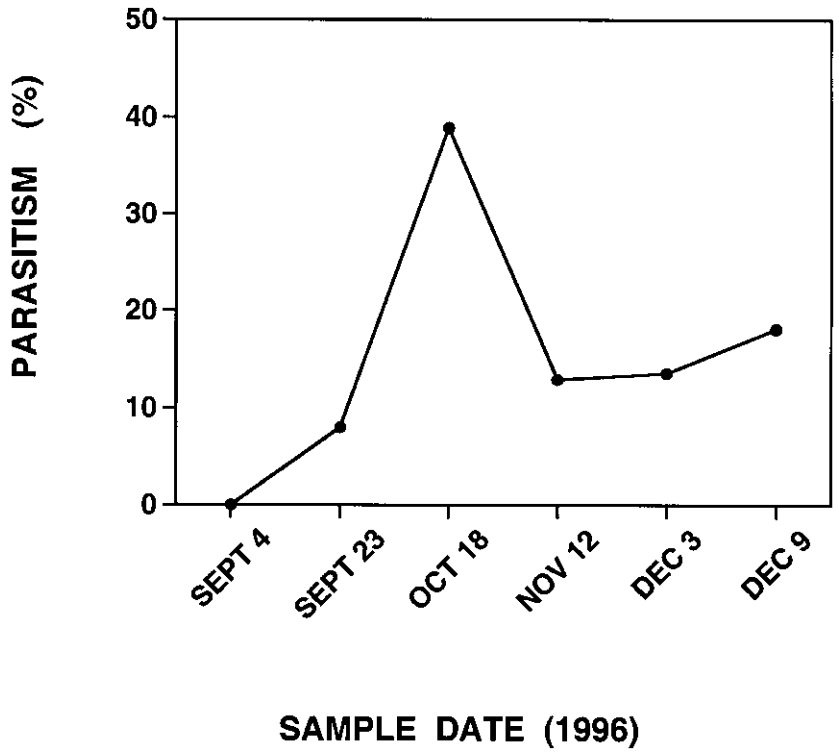

Fig. 7. Overall percentage parasitism by *Ageniaspis citricola* (released in August 1996) of citrus leafminer in the eastern region of the Lower Rio Grande Valley. Recovery samples resulted in up to 39% parasitism in the field.

74.1%, respectively for Feb. 1995; and 23.8°C (74.9°F), 6.2 cm (2.46 in) and 75.6%, respectively for Apr. 1995. Maximum temperatures reached 31.7°C (89°F) in Feb. 1995 and 36.1°C (97°F) in Apr. 1995.

In August 1996, following a release of 626 adult parasite females in the east region of the valley, recovery samples resulted in up to 39% parasitism in the field (Fig. 7). Mean temperature was 29.3°C (84.7°F) and cumulative rainfall was 11.8 cm (4.66 in) in August 1996. Further surveys indicate that *A. citricola* may disperse from the site of release (French & Legaspi 1996). The higher parasitism rates may be due to the more humid conditions in August compared to the earlier releases in February to April. Rainfall also occurred about 10 minutes after the parasites were released. Precipitation is usually highest during the months of August to September in the Lower Rio Grande Valley. In addition, the eastern region is closer to the coast (Gulf of Mexico); conditions are more humid than the central and west parts of the valley. The presence of suitable hosts may also have contributed to the increased incidence of the leafminer parasites (Figs. 2 and 3). Although *A. citricola* were recovered soon after its release in 1996, no recoveries of *A. citricola* were made in 1997. Apparently, the parasites were not able to overwinter successfully. Moreover, high humidity and presence of host eggs and first-instars are essential in survival and reproduction (Edwards and Hoy 1998). Releases of *A. citricola* in Florida have produced promising results, with

some fields yielding almost 100% pupal parasitization during certain periods (Hoy & Nguyen 1994b). Further studies will include continued monitoring of the incidence of citrus leafminer throughout the Lower Rio Grande Valley and assessments of the impact of parasitoids.

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MAYFLIES (EPHEMEROPTERA) OF NORTH CAROLINA AND SOUTH CAROLINA: AN UPDATE

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ABSTRACT

A total of 204 valid species representing 56 genera in 18 families of mayflies are known to occur in North Carolina and South Carolina. Twenty-six of the 182 species of mayflies that are known from North Carolina are reported for the first time. South Carolina has 170 species of mayflies recorded, of which 26 are new state records. Taxonomic changes relevant to the mayfly fauna of both states are discussed.

RESUMEN

Un total de 204 especies válidas que representan 56 géneros en 18 familias de Ephemeroptera se encuentran en los estados de North Carolina y South Carolina. De las 182 especies de Ephemeroptera conocidas de North Carolina, 26 son reportadas por primera vez. South Carolina tiene 170 especies reportadas, de las cuales 26 son reportadas por primera vez. Se discuten los cambios taxonómicos concernientes a la fauna de los Ephemeroptera de ambos estados.

Unzicker and Carlson (1982) listed 184 species representing 45 genera in 16 families of mayflies known in North Carolina and South Carolina. Because they did not

indicate in which state each species occurred, one has to consult the literature to get such information. Since the publication of their list, there have been several taxonomic revisions of mayfly taxa, and species representing new state records and species new to science have been collected from both states. A total of 204 valid species representing 56 genera in 18 families of mayflies have been recorded from North Carolina and South Carolina. The list presented herein indicates that 182 valid species are known from North Carolina compared to 170 species from South Carolina. Twenty-six of the 182 species currently known from North Carolina are reported for the first time. Additionally, the taxonomic status of five unidentified species (*Acentrella* sp, *Amercaenis* sp. *Barbaetis* sp., *Paracloeodes* sp., and *Procloeon* sp.) from North Carolina are being investigated (Table 3). Twenty-six of the 170 species from South Carolina are reported for the first time. The main objective of this paper is to provide aquatic and conservation biologists with the current knowledge of the mayfly fauna of North Carolina and South Carolina, including the pertinent taxonomic and revisionary references for the group.

Significant taxonomic revisions on the supraspecific level are discussed relative to the mayfly composition of North Carolina and South Carolina. Nomenclatural changes of species in various taxa are briefly explained in the endnotes section. Table 2 lists the species that have been reported from North Carolina and South Carolina since the publication of the checklist of Unzicker and Carlson (1982). It also includes species that were published prior to, but were excluded from Unzicker and Carlson's list. Species that represent new unpublished records for each state are listed in Table 3.

BAETIDAE

Recent redefinition of the generic concepts of the family Baetidae (McCafferty & Waltz 1990, 1995; Waltz & McCafferty 1987a, 1987b; Waltz et al. 1985) has drastically changed the composition of the baetid fauna of North Carolina and South Carolina. The six genera (*Baetis, Callibaetis, Centroptilum, Cloeon*, *Heterocloeon,* and *Pseudocloeon*) listed by Unzicker and Carlson (1982) have now increased to 12 genera (*Acentrella, Acerpenna, Baetis, Barbaetis, Callibaetis, Centroptilum, Diphetor, Heterocloeon, Labiobaetis, Paracloeodes, Procloeon,* and *Pseudocentroptiloides*). The genera *Cloeon* and *Pseudocloeon* no longer occur in either state. The only recognized Nearctic species of *Cloeon*, *C. cognatum*, is now known only from the Northeast and Midwest (McCafferty 1996). The *Pseudocloeon* species reported by Unzicker and Carlson (1982) have recently been transferred to the genus *Baetis* except for *P. carolina,* which is now considered a junior synonym of *Acentrella turbida* (McCafferty et al. 1994). Likewise, all the *Centroptilum* species in Unzicker and Carlson's list have been transferred to *Procloeon*. The genus currently has seven and six species known from North Carolina and South Carolina, respectively. *Procloeon pennulatum* was recently reported from North Carolina (McCafferty 1993), but its occurrence in the state is questionable and remains doubtful (Wiersema, pers. comm.). The genus *Centroptilum* is represented in North Carolina by two species: *C. alamance* (= *Neocloeon alamance)* and *C. triangulifer* (= *Cloeon triangulifer*). The latter also occurs in South Carolina (Table 1). See endnotes for more information on the taxonomic changes relative to the baetid fauna of both states.

EPHEMERELLIDAE

Unzicker and Carlson (1982) listed 36 species of Ephemerellidae in North Carolina or South Carolina, but the number has increased to 40 species (Table 1). Based on a

TABLE 1. LIST OF MAYFLIES (EPHEMEROPTERA) FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC).

TAXON	Unzicker & Carlson 1982	NC	SC
ACANTHAMETROPODIDAE			
Acanthametropus pecatonica (Burks)	X°		X
AMELETIDAE			
Ameletus cryptostimulus Carle		X+	X+
A. lineatus Traver	X	X	X
ARTHROPLEIDAE			
Arthroplea bipunctata McDunnough	X	X	X
BAETIDAE[1]			
Acentrella ampla (Traver)	X	X	X
A. turbida (Banks)[2]		X	X
Acentrella sp.		X+	
Acerpenna macdunnoughi (Ide)	X	X	X°
A. pygmaea (Hagen)	X	X	X
Baetis alachua (Berner)	X	X+	X
B. armillatus McCafferty & Waltz	X	X+	X
B. bimaculatus (Berner)	X	X+	X
B. brunneicolor McDunnough	X	X	X
B. cinctutus McCafferty & Waltz		X+	
B. dubius (Walsh)	X	X	X
B. flavistriga McDunnough		X°	X+
B. intercalaris McDunnough	X	X	X
B. pluto McDunnough		X°	X°
B. punctiventris (McDunnough)		X	X
B. tricaudatus Dodds	X	X	X
Barbaetis benfieldi Kennedy		X°	X+
B. cestus (Provonsha & McCafferty)		X+	X+
Barbaetis sp.		X+	
Callibaetis floridanus Banks			X+
C. fluctuans (Walsh)	X	X	X
C. pretiosus Banks	X	X	X
Centroptilum alamance (Traver)	X	X	X°
C. triangulifer (McDunnough)		X+	
Diphetor hageni (Eaton)		X°	X+

Unpublished new state records are marked by a plus sign (+); published records not included in or published since Unzicker and Carlson's (1982) checklist are indicated by (°); endnote numbers indicate notes of taxonomic changes and associated literature sources.

[1]Generic placements in the Baetidae follow McCafferty & Waltz (1990).

[2]*Acentrella turbida* is a senior synonym (McCafferty et al. 1994) of *A. carolina* (Banks).

TABLE 1. (CONTINUED) LIST OF MAYFLIES (EPHEMEROPTERA) FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC).

TAXON	Unzicker & Carlson 1982	NC	SC
Heterocloeon berneri (Müller-Liebenau)			X+
H. curiosum (McDunnough)	X		X
H. petersi (Müller-Liebenau)		X°	
Labiobaetis ephippiatus (Traver)	X	X+	X
L. frondalis (McDunnough)	X	X	X°
L. propinquus (Walsh)	X	X	X
Paracloeodes minutus Daggy		X+	
Paracloeodes sp.		X+	
Procloeon bellum (McDunnough)	X		X
P. fragile (McDunnough)	X	X	X
P. intermediale (McDunnough)	X	X	X
P. pennulatum (Eaton)		X°	
P. quaesitum (McDunnough)	X	X	X
P. rivulare (Traver)		X+	X°
P. rubropictum (McDunnough)	X	X	X
P. rufostigatum (McDunnough)		X+	
P. simile (McDunnough)	X	X	X
P. viridoculare (Berner)	X	X	X
Procloeon. sp.		X+	
Pseudocentroptiloides usa Waltz & McCafferty		X+	
BAETISCIDAE			
Baetisca becki Schneider & Berner		X+	
B. berneri Tarter & Kirchner		X°	
B. carolina Traver	X	X	X
B. gibbera Berner	X	X°	X
B. laurentina McDunnough		X°	
B. obesa (Say)	X	X°	X
B. rogersi Berner	X		X
BEHNINGIIDAE			
Dolania americana Edmunds & Traver	X	X	X
CAENIDAE			
Amercaenis sp.		X+	
Brachycercus berneri Soldán			X°

Unpublished new state records are marked by a plus sign (+); published records not included in or published since Unzicker and Carlson's (1982) checklist are indicated by (°); endnote numbers indicate notes of taxonomic changes and associated literature sources.

TABLE 1. (CONTINUED) LIST OF MAYFLIES (EPHEMEROPTERA) FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC).

TAXON	Unzicker & Carlson 1982	NC	SC
B. flavus Traver			X°
B. maculatus Berner		X+	
B. nitidus Traver	X	X	X
Caenis amica Hagen	X	X	X
C. anceps Traver		X+	
C. diminuta Walker	X	X	X
C. hilaris (Say)	X	X+	X
C. latipennis Banks		X°	X+
C. macafferti Provonsha		X+	X°
C. punctata McDunnough		X°	X°
Cercobrachys etowah Soldán		X+	X+
EPHEMERELLIDAE			
Attenella attenuata (McDunnough)	X	X	X
Drunella allegheniensis Morgan	X	X	X°
D. cornuta (Morgan)	X	X	
D. cornutella (McDunnough)	X	X	X°
D. lata (Morgan)	X	X	X°
D. longicornis (Traver)	X	X	X+
D. tuberculata (Morgan)[3]	X	X	X
D. walkeri (Eaton)	X	X	X°
D. wayah (Traver)	X	X	X°
Ephemerella argo Burks	X	X°	X
E. aurivillii (Bengtsson)		X+	
E. berneri Allen & Edmunds		X°	X°
E. catawba Traver	X	X	X
E. choctawhatchee Berner	X		X
E. crenula Allen & Edmunds	X	X	X+
E. dorothea Needham	X	X	X
E. floripara McCafferty		X+	X°
E. hispida Allen & Edmunds	X	X	X°
E. inconstans Traver	X	X	X
E. invaria (Walker)	X	X	X+
E. needhami McDunnough	X	X°	X
E. rossi Allen & Edmunds	X	X	X

Unpublished new state records are marked by a plus sign (+); published records not included in or published since Unzicker and Carlson's (1982) checklist are indicated by (°); endnote numbers indicate notes of taxonomic changes and associated literature sources.

[3]*Drunella conestee* (Traver) is a southern clinal variant of *D. tuberculata*, and thus a junior synonym (McCafferty 1993).

TABLE 1. (CONTINUED) LIST OF MAYFLIES (EPHEMEROPTERA) FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC).

TAXON	Unzicker & Carlson 1982	NC	SC
E. rotunda Morgan	X	X	X
E. septentrionalis McDunnough	X	X	X
E. subvaria McDunnough	X	X	
Eurylophella aestiva McDunnough		X°	
E. bicolor (Clemens)	X	X	X
E. doris (Traver)[4]	X	X	X
E. enoensis Funk & Sweeney[5]		X°	X°
E. funeralis McDunnough	X	X	X
E. minimella (McDunnough)	X	X	X
E. prudentalis (McDunnough)	X	X+	X
E. verisimilis (McDunnough)	X	X	X
Serratella carolina (Berner & Allen)	X	X	X+
S. deficiens (Morgan)	X	X	X
S. serrata (Morgan)	X	X	
S. serratoides (McDunnough)	X	X	X
S. sordida (McDunnough)	X	X	X+
S. spiculosa (Berner & Allen)	X	X	X+
Timpanoga simplex (McDunnough)[6]	X	X	X
T. lita (Burks)	X	X	X+
EPHEMERIDAE			
Ephemera blanda Traver	X	X	X
E. guttulata Pictet	X	X	X
E. simulans Walker	X	X	X
E. varia Eaton	X	X	X
Hexagenia atrocaudata McDunnough	X	X	X
H. bilineata (Say)	X	X	X
H. limbata (Serville)[7]	X	X	X
H. rigida McDunnough	X	X	X
Litobrancha recurvata (Morgan)	X	X	X
HEPTAGENIIDAE			

Unpublished new state records are marked by a plus sign (+); published records not included in or published since Unzicker and Carlson's (1982) checklist are indicated by (°); endnote numbers indicate notes of taxonomic changes and associated literature sources.

[4]Following Funk & Sweeney (1994), specimens of *Eurylophella* previously identified as *E. temporalis* from east and south of the Appalachians are *E. doris,* and *Ephemerella trilineata* (Berner) is a synonym of *E. doris*.

[5]Nymphs formerly assigned to *Eurylophella coxalis* (McDunnough) are *E. enoensis*; the nymph of *E. coxalis* is unknown (Funk & Sweeney 1994).

[6]*Danella* Edmunds is now recognized as a subgenus of *Timpanoga* (McCafferty & Wang 1994).

[7]*Hexagenia munda* Eaton was synonymized with *Hexagenia limbata* (McCafferty 1984), and their respective subspecies are generally considered ecophenotypes (McCafferty & Pereira 1984, McCafferty 1996).

TABLE 1. (CONTINUED) LIST OF MAYFLIES (EPHEMEROPTERA) FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC).

TAXON	Unzicker & Carlson 1982	NC	SC
Anepeorus simplex (Walsh)			X
Cinygmula subaequalis (Banks)	X	X	X+
Epeorus dispar Traver	X	X	X
E. pleuralis (Banks)	X	X	X°
E. rubidus Traver	X	X	X
E. subpallidus Traver	X	X	
Heptagenia flavescens (Walsh)	X		X
H. marginalis Banks	X	X	X
H. pulla (Clemens)	X	X	X+
H. townesi Traver	X	X	X
Leucrocuta aphrodite (McDunnough)[8]	X	X	X
L. hebe (McDunnough)	X	X	X°
L. juno (McDunnough)	X	X	
*L. maculipennis (*McDunnough)	X		X
L. thetis (Traver)	X	X	X
Macdunnoa brunnea Flowers		X°	X°
Nixe spinosa (Traver)[9]	X	X	
Rhithrogena amica Traver	X	X	X+
R. exilis Traver	X	X	
R. fasciata Traver	X	X	X
R. fuscifrons Traver	X	X	X+
R. pellucida Daggy			X+
R. rubicunda Traver	X	X	
R. uhari Traver	X	X	X
Stenacron carolina (Banks)	X	X	X
S. interpunctatum (Say)[10]	X	X	X
S. pallidum (Traver)	X	X	X+
Stenonema carlsoni Lewis	X	X+	X
S. exiguum Traver	X	X	X
S. femoratum (Say)	X	X	X
S. mexicanum integrum (McDunnough)	X	X	X
S. ithaca (Clemens & Leonard)		X	X

Unpublished new state records are marked by a plus sign (+); published records not included in or published since Unzicker and Carlson's (1982) checklist are indicated by (°); endnote numbers indicate notes of taxonomic changes and associated literature sources.

[8]*Leucrocuta* Flowers was established for the *maculipennis* group of the genus *Heptagenia* Walsh (Flowers 1980).

[9]*Nixe* Flowers was established for the *lucidipennis* and *simplicioides* species-groups of *Heptagenia* (Flowers 1980).

[10]Subspecies of *Stenacron interpunctatum* are no longer recognized (McCafferty & Pereira 1984).

TABLE 1. (CONTINUED) LIST OF MAYFLIES (EPHEMEROPTERA) FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC).

TAXON	Unzicker & Carlson 1982	NC	SC
S. lenati McCafferty		X°	X+
S. meririvulanum Carle & Lewis			X°
S. modestum (Banks)	X	X	X
S. pudicum (Hagen)	X	X	X
S. sinclairi Lewis			X+
S. terminatum (Walsh)	X	X	X
S. vicarium (Walker)	X	X	X°
ISONYCHIIDAE[11]			
Isonychia arida (Say)	X		X
I. bicolor (Walker)	X	X	X
I. georgiae McDunnough		X°	X°
I. notata Traver	X	X	
I. obscura Traver	X	X	
I. sayi Burks			X°
I. serrata Traver	X	X	
I. similis Traver	X	X	X
LEPTOHYPHIDAE			
Leptohyphes dolani Allen	X	X+	X
L. robacki Allen	X		X
Tricorythodes albilineatus Berner	X		X
T. allectus (Needham)	X	X	
T. stygiatus McDunnough	X	X	
LEPTOPHLEBIIDAE			
Choroterpes basalis (Banks)[12]	X	X	X
Habrophlebia vibrans Needham	X	X	X
Habrophlebiodes americana (Banks)	X	X	X
H. brunneipennis Berner	X		X
Leptophlebia austrina (Traver)	X	X	X
L. bradleyi (Needham)		X°	X°
L. collina (Traver)	X	X	X
L. cupida (Say)	X	X	X°

Unpublished new state records are marked by a plus sign (+); published records not included in or published since Unzicker and Carlson's (1982) checklist are indicated by (°); endnote numbers indicate notes of taxonomic changes and associated literature sources.

[11]The following synonyms in *Isonychia* were given by Kondratieff & Voshell (1984): *I. pictipes* Traver is a synonym of *I. arida; I. fattigi* Traver, *I. matilda* Traver, *I. paceolata* Traver, and *I. sadleri* Traver are all synonyms of *I. bicolor; I. annulata* Traver and *I. thalia* Traver are synonyms of *I. georgiae; I. aurea* Traver is a synonym of *I. similis*.

[12]*Choroterpes hubbelli* has been synonymized with *C. basalis* (Banks) (Burian 1995).

TABLE 1. (CONTINUED) LIST OF MAYFLIES (EPHEMEROPTERA) FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC).

TAXON	Unzicker & Carlson 1982	NC	SC
L. grandis (Traver)	X	X	
L. intermedia (Traver)	X	X	X
L. johnsoni McDunnough	X	X	
Paraleptophlebia adoptiva McDunnough	X	X	X°
P. assimilis (Banks)	X	X	X
P. debilis (Walker)	X	X	X°
P. guttata (McDunnough)	X	X	X
P. jeanae Berner	X		X
P. moerens (McDunnough)	X	X	
P. mollis (Eaton)	X	X	X+
P. swannanoa (Traver)	X	X	X
P. volitans (McDunnough)	X	X	X
METRETOPODIDAE			
Siphloplecton basale (Walker)	X	X	X°
S. costalense Spieth		X+	
S. simile Berner			X+
Siphloplecton sp. No.1 Traver[13]	X	X	
NEOEPHEMERIDAE			
Neoephemera purpurea (Traver)	X	X	X
N. youngi Berner	X	X°	X
OLIGONEURIIDAE			
Homoeoneura cahabensis Pescador & Peters		X°	
H. dolani Edmunds, Berner & Traver	X	X°	X
POLYMITARCYIDAE			
Ephoron leukon Williams	X	X	X
Tortopus puella (Pictet)[14]	X	X°	X
POTAMANTHIDAE			
Anthopotamus distinctus (Traver)[15]	X	X	X°
A. verticis (Say)		X+	
PSEUDIRONIDAE			

Unpublished new state records are marked by a plus sign (+); published records not included in or published since Unzicker and Carlson's (1982) checklist are indicated by (°); endnote numbers indicate notes of taxonomic changes and associated literature sources.

[13]Nymphs identified as *Siphloplecton* sp. No. 1 of Traver probably are *S. costalense* (see Berner 1977).

[14]McCafferty (1996) has synonymized *Tortopus incertus* (Traver) with *T. puella* (Pictet).

[15]The genus *Anthopotamus* was established for the North American species previously placed in the genus *Potamanthus* Pictet (McCafferty & Bae 1990).

TABLE 1. (CONTINUED) LIST OF MAYFLIES (EPHEMEROPTERA) FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC).

TAXON	Unzicker & Carlson 1982	NC	SC
Pseudiron centralis McDunnough[16]		X+	X°
SIPHLONURIDAE			
Siphlonurus decorus Traver	X	X	X
S. luridipennis (Burmeister)	X	X	
S. marginatus Traver	X	X	X°
S. mirus Eaton	X	X	X
S. quebecensis (Provancher)	X	X	X

Unpublished new state records are marked by a plus sign (+); published records not included in or published since Unzicker and Carlson's (1982) checklist are indicated by (°); endnote numbers indicate notes of taxonomic changes and associated literature sources.

[16]*Pseudiron meridionalis* Traver is a synonym of *P. centralis* (Pescador 1985).

recent revision of the genus *Eurylophella* (Funk and Sweeney 1994), some species (e.g., *E. coxalis*, *E. lutulenta*, and *E. trilineata*) listed by Unzicker and Carlson do not occur in North Carolina or South Carolina. *Eurylophella lutulenta* was previously reported in North Carolina (Berner 1977, Unzicker & Carlson 1982) but records south of 43°N Latitude are questionable and at least some of the southern records represent *E. enoensis* (Funk and Sweeney 1994). The list of *Ephemerella* species in Unzicker and Carlson (1982) remains the same, except for the addition of *Ephemerella berneri* and *E. floripara*. Both North Carolina and South Carolina have a high species diversity of *Ephemerella*. Taxonomic keys available for identifying nymphs to species, however, must be used with caution. Characters such as submedian abdominal tubercles or protuberances are invariably related to development or growth, and consequently are highly variable and unreliable. Additionally, taxonomic characters such as banding of the legs, and the density and arrangements of abdominal spicules, are confusing unless comparative specimens of the various species are available. A comprehensive revision of the genus is necessary to resolve such taxonomic difficulties.

HEPTAGENIIDAE

The family Heptageniidae is another group that recently has undergone significant taxonomic changes. *Arthroplea bipunctata* and *Pseudiron centralis*, previously placed in the Heptageniidae, have been transferred to Arthropleidae and Pseudironiidae, respectively (McCafferty 1991, Wang & McCafferty 1995). The Nearctic species of *Heptagenia* are now divided into three genera: *Heptagenia*, *Leucrocuta,* and *Nixe* (Flowers 1980) (see endnotes for further information). Four of the 13 Nearctic species of *Heptagenia*, five of the 10 species of *Leucrocuta,* and one of the 15 known *Nixe* species occur in North Carolina or South Carolina. Unzicker and Carlson's (1982) subspecies of *Stenacron interpunctatum* are no longer recognized (McCafferty 1996; McCafferty and Pereira 1984).

TABLE 2. MAYFLY SPECIES THAT HAVE BEEN REPORTED IN THE LITERATURE FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC) BY VARIOUS WORKERS SINCE THE PUBLICATION OF, OR NOT REPORTED IN, UNZICKER AND CARLSON'S (1982) CHECKLIST.

TAXON	SOURCE	NC	SC
BAETIDAE			
Acerpenna macdunnoughi	Carlson (1981)		X
Baetis flavistriga	Lenat & Penrose (1987)	X	
B. pluto	Carlson (1981), Lenat (1983)	X	X
Barbaetis benfieldi	Lenat & Penrose (1987)	X	
Centroptilum alamance	Carlson (1981)		X
Diphetor hageni	Lenat & Penrose (1987)	X	
Heterocloeon petersi	Lenat & Penrose (1987)	X	
Labiobaetis frondalis	Carlson (1981)		X
Procloeon pennulatum	McCafferty (1993)	X	
P. rivulare	Carlson (1977)		X
BAETISCIDAE			
Baetisca berneri	Penrose et al. (1982)	X	
B. gibbera	Lenat & Penrose (1987)	X	
B. laurentina	Lenat & Penrose (1987)	X	
B. obesa	Lenat & Penrose (1987)	X	
CAENIDAE			
Brachycercus berneri	Soldán (1986)		X
B. flavus	Soldán (1986)		X
Caenis latipennis	Provonsha (1990)	X	
C. macafferti	Provonsha (1990)		X
C. punctata	Provonsha (1990)	X	X
EPHEMERELLIDAE			
Drunella allegheniensis	Carlson (1981)		X
D. cornutella	Carlson (1981)		X
D. lata	Carlson (1981)		X
D. walkeri	Carlson (1981)		X
D. wayah	Carlson (1981)		X
Ephemerella argo	Lenat & Penrose (1987)	X	
E. berneri	Carlson (1981), Penrose et al. (1982)	X	X
E. floripara	McCafferty (1985)		X
E. hispida	Carlson (1981)		X
E. needhami	Lenat & Penrose (1987)	X	
Eurylophella aestiva	Funk & Sweeney (1994)	X	
E. enoensis	Funk & Sweeney (1994)	X	X

TABLE 2. (CONTINUED) MAYFLY SPECIES THAT HAVE BEEN REPORTED IN THE LITERATURE FROM NORTH CAROLINA (NC) AND SOUTH CAROLINA (SC) BY VARIOUS WORKERS SINCE THE PUBLICATION OF, OR NOT REPORTED IN, UNZICKER AND CARLSON'S (1982) CHECKLIST.

TAXON	SOURCE	NC	SC
HEPTAGENIIDAE			
Epeorus pleuralis	Carlson (1981)		X
Leucrocuta hebe	Carlson (1981)		X
Macdunnoa brunnea	Flowers (1982)	X	X
Stenonema lenati	McCafferty (1990)	X	
S. meririvulanum	Carle & Lewis (1978)		X
S. vicarium	Carlson (1981)		X
ISONYCHIIDAE			
Isonychia georgiae	Kondratieff &Voshell (1984), Daniel & Morse (1992)	X	X
I. sayi	Kondratieff & Voshell (1984)		X
LEPTOPHLEBIIDAE			
Leptophlebia bradleyi	Carlson (1981), Lenat & Penrose (1987)	X	X
L. cupida	Carlson (1981)		X
Paraleptophlebia adoptiva	Carlson (1981)		X
P. debilis	Carlson (1981)		X
METRETOPODIDAE			
Siphloplecton basale	Carlson (1981)		X
NEOEPHEMERIDAE			
Neoephemera youngi	Lenat & Penrose (1987)	X	
OLIGONEURIIDAE			
Homoeoneuria cahabensis	Lenat and Penrose (1987)	X	
H. dolani	Finn and Herlong (1980)	X	
POLYMITARCYIDAE			
Tortopus puella	Lenat and Penrose (1987)	X	
POTAMANTHIDAE			
Anthopotamus distinctus	Carlson (1981)		X
PSEUDIRONIDAE			
Pseudiron centralis	Berner (1977)		X
SIPHLONURIDAE			
Siphlonurus marginatus	Carlson (1981)		X

TABLE 3. RECENTLY IDENTIFIED MAYFLIES THAT ARE NEW RECORDS FOR NORTH CAROLINA AND SOUTH CAROLINA BUT REMAIN UNREPORTED IN THE LITERATURE. THE SPECIES THAT ARE PRESUMABLY NEW AND UNPUBLISHED ARE LISTED AS "SP."

TAXON	IDENTIFIER	NC	SC
AMELETIDAE			
Ameletus cryptostimulus	B. C. Kondratieff & S. Spichiger	X	X
BAETIDAE			
Acentrella sp.	R. D. Waltz	X	
Baetis alachua	D. Lenat & R. D. Waltz	X	
B. armillatus	D. Lenat & R. D. Waltz	X	
B. bimaculatus	D. Lenat & R. D. Waltz	X	
B. cinctutus	D. Lenat & R. D. Waltz	X	
B. flavistraga	J. B. Glover		X
Barbaetis benfieldi	S. Spichiger		X
B. cestus	D. Lenat & S. Spichiger	X	X
Barbaetis sp.	R. D. Waltz	X	
Callibaetis floridanus	B. C. Kondratieff		X
Centroptilum triangulifer	R. D. Waltz	X	
Diphetor hageni	S. Spichiger		X
Heterocloeon berneri	S. Spichiger		X
Labiobaetis ephippiatus	D. Lenat & R. D. Waltz	X	
Paracloeodes minutus	R. D. Waltz	X	
Paracloeodes sp.	R. D. Waltz	X	
Procloeon rivulare	R. D. Waltz	X	
Procloeon rufostrigatum	N. Wiersema	X	
Procloeon sp.	N. Wiersema	X	
Pseudocentroptiloides usa	N. Wiersema	X	
BAETISCIDAE			
Baetisca becki	D. Lenat & M. L. Pescador	X	
CAENIDAE	A.V. Provonsha	X	
Amercaenis sp.	A.V. Provonsha	X	
Brachycercus maculatus	A. V. Provonsha	X	
Caenis anceps	A. V. Provonsha	X	
C. hilaris	A. V. Provonsha	X	
C. latipennis	J. B. Glover		X
C. macafferti	A. V. Provonsha	X	
Cercobrachys etowah	M. L. Pescador & J. B. Glover	X	X
EPHEMERELLIDAE			
Drunella longicornis	S. Spichiger		X
Ephemerella aurivillii	D. Lenat & M.L. Pescador	X	

TABLE 3. (CONTINUED) RECENTLY IDENTIFIED MAYFLIES THAT ARE NEW RECORDS FOR NORTH CAROLINA AND SOUTH CAROLINA BUT REMAIN UNREPORTED IN THE LITERATURE. THE SPECIES THAT ARE PRESUMABLY NEW AND UNPUBLISHED ARE LISTED AS "SP."

TAXON	IDENTIFIER	NC	SC
E. crenula	S. Spichiger		X
E. floripara	M. L. Pescador	X	
E. invaria	S. Spichiger		X
E. prudentalis	D. Lenat & D. H. Funk	X	
Serratella carolina	J. B. Glover		X
S. sordida	J. B. Glover		X
S. spiculosa	J. B. Glover		X
Timpanoga lita	S. Spichiger		X
HEPTAGENIIDAE	S. Spichiger		X
Cinygmula subaequalis	S. Spichiger		X
Heptagenia pulla	M. K. Griffin		X
Rhithrogena amica	S. Spichiger		X
R. fuscifrons	S. Spichiger		X
R. pellucida	B. C. Kondratieff		X
Stenacron pallidum	M. K. Griffin		X
Stenonema carlsoni	M. L. Pescador	X	
S. lenati	S. Spichiger		X
S. sinclairi	S. Spichiger		X
LEPTOHYPHIDAE			
Leptohyphes dolani	D. Lenat	X	
LEPTOPHLEBIIDAE			
Paraleptophlebia mollis	S. Spichiger		X
METRETOPODIDAE			
Siphloplecton costalense	D. Stephan	X	
S. simile	B. C. Kondratieff		X
PSEUDIRONIDAE			
Pseudiron centralis	M.L. Pescador	X	
POTAMANTHIDAE			
Anthopotamus verticis	W. P. McCafferty	X	

ISONYCHIDAE AND OLIGONEURIIDAE

The recent resurrection of the monogeneric family Isonychiidae (McCafferty 1991a,b) has reduced the species composition of the oligoneuriid fauna of both states. Following Kondratieff and Voshell's (1984) revision of the genus *Isonychia,* a total of eight species are now known from North Carolina and South Carolina compared to the previous 15 species reported by Unzicker and Carlson (1982). The oligoneuriids

Homoeoneuria dolani and *H. cahabensis* both occur in North Carolina, with the former species also occurring in South Carolina.

POTAMANTHIDAE

The potamanthid *Anthopotamus verticis* has recently been reported from North Carolina (McCafferty 1994), increasing to two the number of *Anthopotamus* species known from that state; the other species is *A. distinctus*, which also occurs in South Carolina.

SIPHLONURIDAE, ACANTHAMETROPODIDAE, AND AMELETIDAE

Some genera and species that were listed in the Siphlonuridae by Unzicker and Carlson (1982) are no longer considered to belong to the family. The genus *Acanthametropus*, which is now placed in the family Acanthametropodidae, has one species, *A. pecatonica*, occurring in South Carolina. The genus *Ameletus* (Ameletidae) is represented in North and South Carolina by *A. cryptostimulus* and *A. lineatus* (Table 1).

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DESCRIPTION OF THE FINAL INSTAR LARVA OF *PERITHOUS SCURRA* WITH COMMENTS ON ITS MORPHOLOGICAL CHARACTERS (HYMENOPTERA: ICHNEUMONIDAE, PIMPLINAE, DELOMERISTINI)

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ABSTRACT

A description is given of the final instar-larva of *Perithous scurra* (Panzer, 1804), which is compared with the previously described species of the genus, *P. divinator* (Rossius, 1790). The greater or lesser development of the epistoma, and the number of sensilla of the maxillary and labial palpi are the best characters for distinguishing between both species. Additionally, the larva of *P. scurra* presents spinules on the tegument and sensilla on the antennal orbits, but none of those structures has been described in *P. divinator*.

Key Words: Ectoparasitoid, final-instar larva, Ichneumonidae

RESUMEN

Se describe la larva madura de *Perithous scurra* (Panzer, 1804), y se compara con la especie previamente descrita del género, *P. divinator* (Rossius, 1790). El mayor o menor desarrollo del epistoma, y el número de sensilas de los palpos maxilares y labiales, son los caracteres que mejor permiten distinguir ambas especies. Adicionalmente, la larva de *P. scurra* presenta espínulas en el tegumento y sensilas en las órbitas antenales, aunque ninguna de estas estructuras ha sido citada en *P. divinator*.

The immature instars of parasitic Hymenoptera have been studied by several authors, the classic works of Clausen (1940) and Hagen (1964) being outstanding, as are the keys for the taxonomic differentiation of mature larvae compiled by Beirne (1941), Short (1952, 1959, 1970, 1976), Finlayson (1967, 1975) and Capek (1970). Within this broad group, the Ichneumonidae have been particularly studied by Short (1978), Finlayson & Hagen (1979), Finlayson (1967, 1987), Wahl (1986), and Brooks & Wahl (1987).

Here we describe the mature larva of a species of Pimplinae (= Ephialtinae, sensu Townes, 1969) [Delomeristini (sensu Fitton et al., 1988)] [= Theronini auct.]: *Perithous scurra* (Panzer, 1804) (= *mediator* Fabricius). This species is a larval ectoparasitoid of Hymenoptera Aculeata that nest in hollow stems (Aubert, 1969), and its biology has been studied by Verhoeff (1891), Bouché (1847), Brocher (1926), and Fitton et al. (1988). Although a detailed description of the internal anatomy of the mature larva was provided by Brocher (1926), he failed to describe the morphological characters present on the cephalic structures (sclerotized areas of the external cephalic skele-

ton), spiracles (usually prothoracic), and tegument, that would permit a characterization of the preimaginal stages of Hymenoptera Parasitica (Short 1978; Finlayson & Hagen 1979).

MATERIALS AND METHODS

Three mature larvae and six imagos of *P. scurra* were obtained from two trap nests of *Ailanthus altissima* Swingle (Simaroubaceae), in which *Pemphredon lethifer* (Shuckard, 1837) (Hymenoptera, Sphecidae) had nested. The trap nests were placed in the field in April 1993 at Casas Valle Carmona (Cuenca, Spain) and were collected in September. The methodology of Asís (1990) was employed to open the nests, and for data collection, preservation, and preparation; the terminology of Finlayson (1987) was used for the description of the mature larva.

Voucher specimens are deposited in the Departamento de Zoología, Facultad de Biología, Universidad de Salamanca, Salamanca (Spain).

RESULTS

Perithous scurra (Panzer)

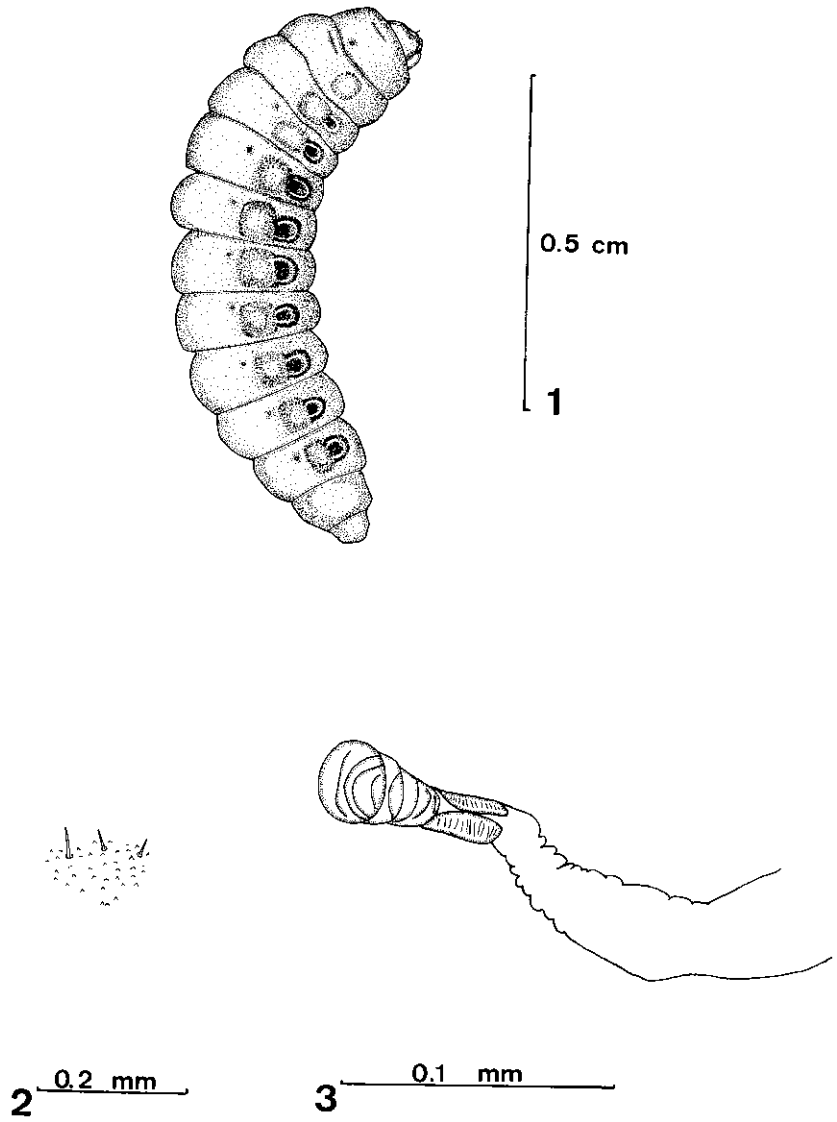
Of the eight cells contained in one of the nests of *P. lethifer*, the externalmost five were parasitized; in the other nest with nine cells the externalmost three and the sixth were parasitized. The mature larva had not constructed a cocoon in any case. One male and two females of the parasitoid emerged from the first of the nests (two mature larvae were stored in vials with 70% ethanol for later study), while three females emerged from the second one (one larva was preserved for later study). Two of the larvae (ref.: 93070401) were prepared for microscopic examination while the other one (ref.: 93070402 A) was used for whole-body drawings.

Mature larva. *General aspect* (Fig. 1). Elongate (length = 1.25 cm, maximum width = 22 mm), more or less fusiform, slightly curved body, with three thoracic and ten abdominal segments; whitish, weakly sclerotized, except spiracles and setae. Anus small, almost terminal. Pleural lobes well developed. Tegument papillose, with spinules (l = 3 μm) and setae (l = 25-40 μm) (Fig. 2); the latter in a transverse line around each of the body segments.

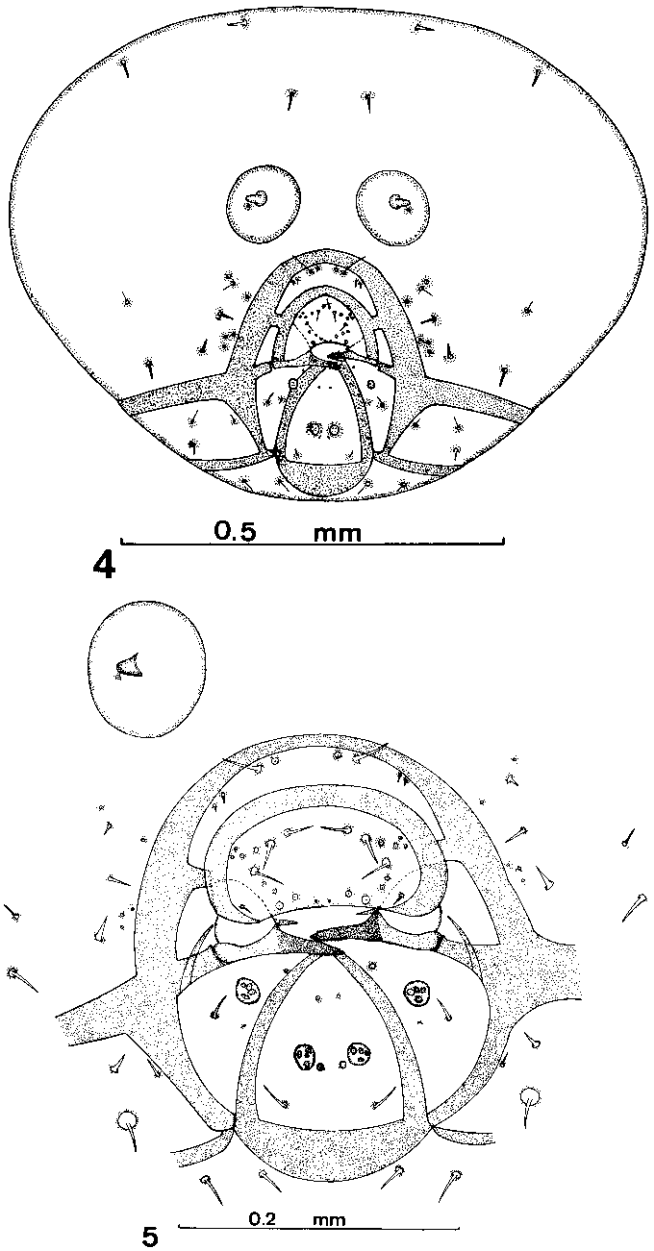
Spiracles (Fig. 3) located on prothorax and first eight abdominal segments; atrium (diameter = 30 μm) round, unarmed, separated from closing apparatus (l = 22 μm, w = 15 μm) by a short section of trachea (l = 19 μm, w = 18 μm).

Cranium (Fig. 4) (w = 825 μm, h = 630 μm) small, weakly sclerotized, slightly retracted within prothorax; with sensilla (d = 6 μm) and sparse setae (l = 13-50 μm), more numerous on lateral side of sclerotized areas of external cephalic skeleton; ecdysial suture line and ocular lines undifferentiated (they are slightly apparent on larva not treated for microscopy); antennae papilliform (l = 32 μm; w =25 μm), with a sensillum (d = 5 μm) on antennal orbit; epistoma completely developed; pleurostoma, hypostoma, lacinial sclerite, hypostomal spur and stipital sclerite well sclerotized; superior and inferior pleurostomal processes present; cardo absent; ventral zone of labial sclerite twice or three times width of lateral zones; labral sclerite complete, well sclerotized; clypeus with six setae (l = 5-23 μm) and two sensilla (d = 4 μm); labrum with eight setae (l = 18-20 μm) and eighteen sensilla (l = 1-5 μm).

Mouthparts (Fig. 5). Mandibles with wide base and blade bifurcate; in this bifurcated blade, the larger part has teeth on both dorsal and ventral surfaces, the smaller one being unarmed, and with a large posteromedial tooth; maxillae with five setae (l



Figs. 1-3. Mature larva of *P. scurra* (Panzer). 1. Lateral view of body; 2. Detail of tegument with papilla, spinules and setae; 3. Spiracle (atrium, trachea, spiracular closure).



Figs. 4 & 5. Mature larva of *P. scurra* (Panzer). 4. Cranium (frontal view); 5. Detail of antenna, sclerotized areas of cranium (the hypostoma has been cut off), clypeus, labrum and mouthparts.

= 3-20 μm); labium with four setae (l = 3-20 μm); maxillary (w = 15 μm) and labial (w = 16 μm) palpi not protruding, disc shaped with four sensilla on each; salivary orifice transverse ovoid; spinneret undifferentiated; postlabium with four setae (l = 20 μm).

Discussion

The results obtained in the present work confirm the data reported by Wolf (1953) and Fitton et al. (1988) concerning the hosts and the parasitic behaviour of *P. scurra.* According to Fitton et al. (1988), at least the British species of the genus *Perithous* Holmgren, 1859 would have become specialist idiobiont ectoparasitoids of sphecids, parasitizing their prepupal stage. In Great Britain, *P. scurra* parasitizes *Pemphredon lugubris* (Fabricius, 1793), pupating inside the cell of its host without constructing a cocoon. Although these authors consider *P. lugubris* as a usual host of *P. scurra*, Wolf (1953) obtained this pimpline from *P. lethifer.*

As in the case of the larvae of the Pimplinae (Short 1978; Finlayson 1967, 1975, 1987), the mature larva of *P. scurra* has the hypostomal spur joining the stipital sclerite very close to where the latter joins the labial sclerite. As in the other Delomeristini, the characters described by Short (1978) are well defined; and as an ectoparasitoid species the mandible blades have teeth and a broad unarmed projection in the form of a tooth on the base and blade of the mandibles, nine pairs of functional spiracles (one prothoracic and eight abdominal), and an undifferentiated spinneret.

Unlike the previously described species of the genus, *P. divinator* (Danks 1970; Short 1978), *P. scurra* displays the following traits (Table 1): a) more setae on clypeus, maxilla, and postlabium; and more sensilla on clypeus and labrum; b) completely developed epistoma [a character only present, among the Delomeristini, in *Pseudorhyssa* Merrill, 1915 (Short 1978)]; c) maxillary and labial palpi with four sensilla (character present in only this species among Delomeristini); d) undifferentiated spinneret [in *P. divinator*, although not very differentiated, the spinneret is perceptible]; e) tegument with spinules [this character was not reported either by Danks (1970) or Short (1978) for *P. divinator*]; and f) antennal orbit with a single, well differentiated sensillum.

Furthermore, in the species studied the pleurostoma is well sclerotized, as in *P. divinator* and *Pseudorhyssa*, and there is no seta on the posterior part of the stipital sclerite, the cardo being absent. These latter two characters were reported by Short (1978) for *P. divinator*, although Danks (1970) did not include them in his description.

In any case, it should be noted that the comparison has been made with Short's (1970) and Dank's (1978) descriptions of *P. divinator*; according to Brooks & Wahl (1987), those descriptions could be inadequate in several aspects.

Table 1. Morphological differences between the mature larvae of *P. Scurra* (Panzer) and *P. Divinator* (Rossi). Characters [Presence (*); Absence (-)]: Number of Setae On: 1) Clypeus; 2) Maxillae; 3) Postlabium; Number of Sensilla On: 4) Clypeus; 5) Labrum; 6) Maxillary and Labial Palpi; 7) Epistoma Completely Developed; 8) Spinneret; 9) Spinules On Tegument; 10) Sensillum Of Antennal Orbit.

Species	1	2	3	4	5	6	7	8	9	10
P. scurra	6	6	4	6	18	4	*	—	*	*
P. divinator	4	3/4	2	4	2	3	—	*	?	?

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DEFENSE OF OVIPOSITION SITES BY FEMALE ORIENTAL FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Field observations revealed that females of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), defended oviposition sites on mangos (*Mangifera indica* L.) against conspecific females. In most encounters, females simply lunged at opponents and chased them off the fruit without physical contact. However, head-butting and pushing were observed in about 10% of the contests. Body size was a key determinant of fighting success, with larger females winning 85% of the encounters. In a field experiment, arrivals, oviposition, and aggression of females were compared between intact vs. sliced peaches. Similar numbers of females landed on the two classes of fruits, but a greater proportion of alighting females oviposited on sliced peaches than intact peaches. The adaptive function of female territoriality is discussed in light of these findings.

Key Words: fruit fly, aggression, territory, oviposition, Hawaii

RESUMEN

Observaciones de campo han indicado que las hembras de la mosca oriental de la fruta, *Bactrocera dorsalis* (Hendel), defienden los sitios de oviposición en mangos (*Mangifera indica* L.) de las hembras de su misma especie. En la mayoría de los encuentros, las hembras simplemente se lanzaron hacia sus oponentes y las espantaron de la fruta sin tener contacto físico directo. Sin embargo, choques de cabeza y empujones fueron observados en el 10% de los encuentros. El tamaño del cuerpo es clave en la determinación del éxito en las luchas; éste se refleja en que las hembras más grandes ganaron en un 85% de los encuentros. En un experimento de campo, llegadas, ovi-

posición, y comportamiento agresivo de las hembras fue comparado entre melocotones intactos y partidos. Un número similar de hembras visitaron las 2 clases de frutas, pero una proporción mayor de las hembras ovipositó en los melocotones partidos en comparación con los intactos. La función adaptativa de la territorialidad de las hembras se discute en base a estos hallazgos.

Territorial behavior has been reported for males of many insect species (Baker 1983, Fitzpatrick & Wellington 1983). In most of these cases, males defend sites containing resources vital to females, thus increasing their mating opportunities. Though less common, territorial behavior has also been reported for females in several insect species, and in these instances site defense is usually related to food resources. For example, female water striders defend particular areas of streams where food resources collect (Vepsalainen 1985, Nummelin 1988), and female aphids defend basal sections of newly developing leaves (Whitham 1979).

In tephritid fruit flies, territorial behavior has been reported frequently for males. Male defense of mating areas has been described for various species of *Rhagoletis* (Boyce 1934, Prokopy & Roitberg 1984), *Anastrepha* (Aluja 1994), and *Bactrocera* (Fletcher 1987) as well as for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Arita & Kaneshiro 1989). In contrast, there are few reports of female-female aggression in tephritids, and these are typically based on observations made in the laboratory. For example, Biggs (1972) and AliNiazee (1974) described several types of agonistic displays for females of *R. pomonella* (Walsh) and *R. indifferens* (Curran), respectively, caged under high experimental densities. In the wild, however, aggression between *Rhagoletis* females may occur only infrequently (Prokopy & Bush 1973). Similarly, McDonald & McInnis (1985) noted fights between *C. capitata* females at potential oviposition sites, but again these occurred under artificially high densities in the laboratory. An instance of interspecific aggression (between *A. obliqua* (Macquart) and *C. capitata* females) has also been reported in laboratory observations (Camargo et al. 1996).

The apparent low incidence and intensity of female aggression in *Rhagoletis* and *Ceratitis* (as well as *Anastrepha*) may reflect the use of host marking pheromones by ovipositing females in these genera (Fletcher & Prokopy 1991). Following egg-laying, females drag their ovipositor over the fruit surface and deposit a pheromone that tends to deter future oviposition in that fruit by conspecific females (Fletcher & Prokopy 1991). Thus, in these species interference between females may be primarily via chemical signals, with active site defense (involving physical displays or contact) being less important.

Interestingly, host marking pheromones are apparently absent in *Bactrocera* females (Fletcher & Prokopy 1991), and, based on one study at least, inter-female aggression may correspondingly be more frequent and intense in these species. In his study of *B. tryoni* (Froggatt), Pritchard (1969) reported that females on fruit, even those already engaged in egg-laying, were easily disturbed by intruders and made threat displays that occasionally escalated to head-butting and pushing. Nearly 20% of females observed ovipositing in the wild interrupted egg-laying to drive off conspecific females. Despite female aggression, Pritchard (1969) found that eggs were not uniformly spaced, as might be expected, but were highly aggregated both among and within fruits.

The present paper supplies information on female defense of oviposition sites in the oriental fruit fly, *Bactrocera dorsalis* (Hendel). Field observations provided data on the incidence and intensity of female aggression on host fruits, and a field experi-

ment was performed to examine the influence of fruit wounds on the occurrence of oviposition and female-female fighting. The function of female territoriality in this species is discussed in light of the present findings.

Materials and Methods

Field Observations

Behavioral observations were made at a single mango tree (*Mangifera indica* L.) in a vacant lot in Honolulu, Hawaii, during June 1993. The tree (approximately 10 m high) bore a large crop but had already dropped many ripe fruits. Female *B. dorsalis* were abundant on the fallen mangos, and all observations were made on these fruits. Data were gathered by 1-3 observers between 1200-1400 hours on 5 sunny days with air temperatures ranging between 28 and 31°C.

Individual females were observed continuously or until lost from view. Observations were recorded on tape for later transcription, and the following information was noted: total observation time, number of different mangos visited, time spent on a given mango (underestimates in many cases, because females were already on fruits for unknown time intervals when observations commenced), number and duration of oviposition bouts (operationally, oviposition was equated with ovipositor-boring into a fruit), number of different oviposition sites on a given fruit, presence of a conspicuous hole or gash (in fruit surface) at the oviposition sites, number and outcome of aggressive interactions with conspecific females. I also classified fruit departures and oviposition stoppages either as unprovoked (without apparent cause) or as resulting from aggression.

To determine whether fighting ability was size-dependent, agonistic encounters between *B. dorsalis* females were observed on 6 additional dates at the same site, and the participants were collected for body size measurements. As an index of overall size, the length of the posterior edge of the discal cell was measured to the nearest 0.1 mm using a dissecting microscope equipped with a disc micrometer. The female remaining on the fruit after aggression was identified as the winner, and the departing female was considered the loser. Females were also classified as resident (individual initially seen on fruit) or intruder.

Field Experiment

Work was conducted in July 1993 at the University of Hawaii Agricultural Experiment Station in Waimanalo, Oahu, Hawaii. Experimental fruits were placed on the ground beneath a row of large mango trees. Many ripe mangos were on the ground, and female *B. dorsalis* were seen ovipositing on these fruits. Males were not observed on these fruits. Data were gathered by 1-3 observers between 1100-1300 hours on 5 sunny days with air temperatures ranging from 30 to 33°C.

Studies on *C. capitata* (Papaj et. 1989) and *B. tryoni* (Oi & Mau 1989) have demonstrated that ovipositing females prefer fruits with wounds or holes over intact fruit, presumably to facilitate egg-laying. Accordingly, the field study compared female arrivals, oviposition, and aggression on intact vs. sliced groups of peaches (*Prunus persica* L.). Peaches were placed on the ground at 1100 hours and observed continuously over the next 2 h. Store-bought California peaches were used to avoid any prior infestation and to insure size uniformity. The fruits were washed and dried prior to use. Peaches in the intact group were not modified in any way, while those in the sliced group received a single cut (4-5 cm long; 1-2 cm deep) immediately before observa-

tions. A single observer monitored 4-6 fruits simultaneously (placed 0.6-0.75 m apart in the shade) and recorded the following information on tape for later transcription: number of female arrivals, duration of female residency, total duration of oviposition activity, site of oviposition (cut vs. smooth surface in sliced fruits), and the number and outcome of aggressive encounters. As before, departures from fruit and termination of egg-laying were categorized as unprovoked or as a consequence of aggression.

Statistical Analysis

Means were compared using the nonparametric Mann-Whitney test to avoid assumptions of normality, though variation about means was described using the standard deviation (SD). Contingency tests were performed using the G test (log likelihood ratio test) with Yates' correction for continuity. The normal approximation to the binomial test was used to test for resident advantage in aggression. Computational procedures followed Zar (1974).

RESULTS

Field Observations

Over the entire study, 137 observations were made ranging from 0.2 to 33.3 min in length and totalling approximately 14 h. Females usually (119/137) remained on a single fruit during observations, but in some cases they visited 2-5 fruits. Thus, the total number of fruits visited (n = 164 over all females) exceeded the number of observations made (n = 137). Females were seen ovipositing during nearly 1/3 (39/137) of the observations.

The length of female residency on a fruit was dependent on her oviposition activity. On average, females that did not oviposit on a given fruit stayed for 136 s (SD = 159, n = 125). By comparison, females that laid eggs on a given fruit remained for an average of 732 s (SD = 452, n = 39; $P < 0.001$; Mann Whitney test). Females that oviposited did so for an average of 35% (SD = 23, n = 39) of their time on a given fruit. In most cases (25/39), females oviposited, not in a single episode, but during multiple bouts on the same fruit. Females often interrupted oviposition temporarily, walked around the fruit, and then returned to the original area for further egg-laying. On average, females seen ovipositing did so over 2.5 bouts (SD = 2.0, n = 39), and each bout lasted 1.65 min (SD = 1.4, n = 99). In almost all cases (34/39), eggs were laid at only one site on a given fruit, and most of these sites (27/39) had either a visible hole or gash in the fruit surface.

The incidence of aggression was related to the oviposition activity of females. During visits in which no oviposition occurred, females rarely engaged in aggression with other females (14/125). In contrast, females that oviposited on a given fruit interacted aggressively with conspecific females in 1/2 of the instances (19/39; $P < 0.001$; G test). At first glance, this difference appears to have been directly related to residency time on a fruit: visits with oviposition were, on average, about 5.5 times longer than those without oviposition (732 s vs. 136 s, respectively) and were about 4.5 times as likely to be accompanied by agonistic encounters as those without oviposition (49 vs. 11%, respectively). However, data regarding the rate of fighting reveal that, independent of residency duration, aggressive encounters occurred far more frequently on fruits on which oviposition was observed. On average, the number of aggressive encounters occurring per minute was nearly 5 times greater during visits with oviposition (x=0.19/min, n = 39) than those without oviposition (x = 0.04/min; n = 125; $P < 0.001$; Mann Whitney test). Resident females detected most intruders, and only 10% (10/101) and

15% (2/13) of intruding females went unnoticed by ovipositing and non-ovipositing females, respectively ($P > 0.05$; G test). Intruders in these cases usually stayed less than 20 s and departed on their own volition.

Consistent with these data, losing an agonistic encounter was a more likely cause of female departure from fruits where the female oviposited than from fruits where she did not. In 1/3 (13/39) of the visits during which oviposition occurred, the resident female left the fruit immediately after fighting with an intruding female (who invariably remained on the fruit). In contrast, in visits without oviposition female departures were usually unprovoked and only infrequently (14/125) followed aggression ($P<0.001$; G test). In addition, female-female aggression also limited the duration of individual oviposition bouts: 37% (37/99) of egg-laying bouts ended when the resident female detected and subsequently fought with an intruding female.

In most of the aggressive encounters observed (90/102), there was no physical contact between the participants. Females extended their legs, thus elevating their body, and held their wings perpendicular to their body. In most of these cases (70/90), one female simply lunged at the other and chased it away. In the remaining observations, the combatants moved back and forth in front of each other for several seconds prior to a chase. When aggression escalated to physical contact (12 cases, all on fruits where oviposition was observed), females ran directly toward one another and butted heads from 1 to 10 consecutive times in interactions lasting 1-63 s. In the longer contests, head butting occurred while the antagonists circled closely about one another, with each trying to push the other off the fruit. Actual wrestling was observed in 3 instances, and in each case the females fell to the ground where they continued grappling for 2-5 s.

Body size was a key determinant of fighting success. Body size measurements were obtained for 41 chases (physical contact absent), and larger females won 85% of the interactions (32/37; combatants were the same size in 4 cases; $P < 0.001$; binomial test). Residency appeared to be unimportant in determining the outcome of agonistic encounters: the proportion of contests won by residents (23/41) did not differ significantly from 50% ($P > 0.05$; binomial test).

Field Experiment

On average, similar numbers of females landed on intact ($\bar{x} = 3.4$, $SD = 3.0$) and sliced peaches ($\bar{x} = 3.5$, $SD = 3.3$, $n = 52$ for both groups; $P > 0.05$; Mann Whitney test). However, the proportion of females that subsequently oviposited was greater for sliced (65/184) than intact (21/174) fruits ($P < 0.001$; G test). As these data suggest, females deposited eggs in a greater proportion of sliced peaches (38/52) than intact ones (16/52; $P < 0.001$; G test).

As before, residency time was related to the incidence of egg-laying. On the sliced peaches, females that oviposited spent an average of 894 s ($SD = 778$, $n = 65$) on a given fruit compared to only 141 s ($SD = 100$, $n = 119$) for non-ovipositing females ($P<0.001$; Mann Whitney test). Similarly, on the intact peaches, females that oviposited spent an average of 828 s ($SD = 699$, $n = 21$) compared to only 129 s ($SD = 116$, $n=153$) for non-ovipositing females ($P < 0.001$; Mann Whitney test). Also, consistent with the behavioral observations described above, aggression on both sliced and intact fruits usually involved females seen to oviposit. On sliced peaches, agonistic encounters were observed during about 50% of the visits involving ovipositing females (31/65) compared to only 5% (6/119) for non-ovipositing females ($P < 0.001$; G test). On intact peaches, aggression was noted in approximately 25% (5/21) of the visits made by females that oviposited compared to only 3% (4/153) of those visits in which females did not oviposit ($P < 0.001$; G test).

Among females that oviposited, mean residency time did not differ significantly between the two types of fruit ($n_1 = 65$, $n_2=21$; $P > 0.05$; Mann Whitney test). However, females on sliced peaches spent, on average, more time engaged in egg-laying than did those on intact peaches (366 vs. 192 s, $n_1 = 65$, $n_2 = 21$; $P < 0.01$; Mann Whitney test). The incidence and frequency of aggression also differed between females ovipositing on the two fruit types. Despite similar residency periods, females ovipositing on sliced peaches were more likely to have an agonistic interaction than those ovipositing on intact ones (31/65 vs. 5/21, respectively; $P < 0.001$; G test). In addition, fights occurred more frequently on sliced peaches ($x = 0.07$/min, SD = 0.04, $n = 65$) than on intact ones ($x = 0.03$/min, SD = 0.02, $n=21$; $P < 0.001$; Mann Whitney test). As before, resident females nearly always detected intruders, and only 7% (6/59) and 10% (1/10) of intruders arrived (and subsequently departed) unnoticed on sliced and intact peaches, respectively ($P > 0.05$; G test).

Discussion

The aggressive behavior of *B. dorsalis* females appears very similar to that described for females of *B. tryoni* (Pritchard 1969). Females of both species responded quickly to the presence of intruders and interrupted egg-laying to confront intraspecific females. Though no data were presented, Pritchard (1969) noted that in *B. tryoni* visual displays were "usually effective in causing intruders to leave", and likewise contests between *B. dorsalis* females rarely (10% of interactions) involved bodily contact. Escalation in both species involved episodes of repeated head butting, and, if this was unsuccessful, prolonged pushing resulted.

As noted in *B. tryoni* (Pritchard 1969; Eisemann & Rice 1985) and *C. capitata* (Papaj et al. 1989), females of *B. dorsalis* preferentially oviposited in existing holes or cuts in the fruit surface (see also Oi & Mau 1989). Evidence for this preference derived from the field experiment where alighting females were found to be approximately 3 times more likely to oviposit on sliced peaches than intact ones. In addition, the great majority of natural oviposition occurred in fruit wounds. Choosing existing holes for oviposition presumably facilitates the physical actions associated with egg-laying and enhances egg and larval survival (Papaj et al. 1989).

Based again on the field experiment, both the incidence and frequency of female defense were higher on sliced fruits than on intact fruits. This trend resulted directly from the higher numbers of intruders alighting on the sliced peaches: intruders arrived twice as frequently on sliced peaches as intact ones. Interestingly, although more intruders arrived at sliced peaches, there was no difference in the numbers of females alighting on unoccupied sliced and intact peaches (see Prokopy et al. [1990] for contrary results). Thus, the combination of alighted female plus surface cut was apparently more attractive to searching females than either a surface cut (on an unoccupied fruit) or a female (on an intact fruit). The difference in aggression levels between fruit types did not result from variation in female vigilance: residents of both sliced and intact peaches detected and interacted with nearly all intruders. Thus, there was no evidence to suggest that females defended sliced peaches more readily than intact ones.

The field observations are consistent with the findings of the field experiment. At the mango tree, oviposition usually occurred in existing holes or cuts in the surface of the fruits. Whether or not the distribution of oviposition among mangos reflected the distribution of suitable holes or wounds is unknown but appears likely. The incidence and mean arrival rate of intruders was also greater during visits where oviposition was observed than visits where oviposition was absent. The propensity of females to oviposit in fruit wounds suggests again that searching females are highly attracted by the co-occurrence of a female and a surface cut.

The adaptive function of female territoriality in *Bactrocera* is presumably related to its effects on larval competition. By defending fruits (even temporarily), females may provide their larvae with a "head start" in growth over unrelated larvae and hence a competitive advantage for host fruit resources. Interestingly, ovipositing *B. dorsalis* females are apparently unable to detect unhatched, conspecific eggs within host fruit and do not discriminate against egg-laden fruits in selecting oviposition sites (Prokopy et al. 1989). This same study showed, however, that females discriminated against fruit containing conspecific (or heterospecific) larvae. Thus, by limiting the opportunity for larval food competition and facilitating growth of its own larvae, aggressive behavior may also serve to deter oviposition (via larval detection) by other females well after the original territorial female has departed.

Female preference for existing holes and their inability to detect and avoid egg-laden fruit may collectively limit the potential advantages of territoriality. Defense of an already infested (i.e., egg-laden) fruit or a fruit soon likely to receive additional eggs (i.e., prior to the hatching of a female's own eggs) would probably not confer significant benefits, since larval competition would not be much reduced. Females do not have perfect information about potential oviposition sites, and this uncertainty jeopardizes the value of their aggressive actions. Still, the potential benefits to larval growth may outweigh the actual costs of site defense, which appear trivial in terms of both time and energy expenditure and risk of injury. In short, territorial behavior most likely has probabilistic benefits; it may confer a fitness advantage, but it does not guarantee one. Therefore, identifying the environmental influences (e.g., population density, host fruit) on the benefits conferred by territoriality is a key step toward elucidating the adaptive value of this behavior.

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DESCRIPTION OF *ANASTREPHA SORORCULA* AND *A. SERPENTINA* (DIPTERA: TEPHRITIDAE) EGGS

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ABSTRACT

The morphology of *Anastrepha sororcula* Zucchi and *A. serpentina* (Wiedemann) eggs are described by scanning electron microscopy. *A. sororcula* eggs present a conspicuous sculpturing of the chorion at the micropylar end while the eggs of *A. serpentina* are devoid of such ornamentation. The sculpturing of the *A. sororcula* eggs, represented by ridges in a polygonal arrangement, is more developed in the ventral than in the dorsal side of the egg. The micropyle is located in the dorsal side near the egg apex and is surrounded by a row of chorionic polygons. In *A. serpentina* the micropyle is located in a prominent rim very near the extremity of the egg. Aeropyles are found at the anterior end usually at the ventral side of the eggs. Fractured eggs of both species showed that the chorion is cavernous throughout the egg body. It is suggested that these eggs may be able to perform a plastron-mediated respiration. The results suggest that egg morphology may be useful to elucidate taxonomic and phylogenetic relationship among *Anastrepha* species.

Key Words: Fruit flies, chorion, eggshell, plastron

RESUMEN

La morfología de los huevos de *Anastrepha sororcula* Zucchi y de *A. serpentina* (Wiedemann) es descrita por microscopía electrónica. El corion de los huevos de *A. sororcula* presenta una escultura conspicua en el extremo micropilar mientras que los huevos de *A. serpentina* están desprovistos de tal ornamentación. La ornamentación de los huevos de *A. sororcula*, representada por pliegues en un arreglo poligonal, está más desarrollada en el lado ventral que en el lado dorsal. El micrópilo se localiza en el lado dorsal cerca del ápice del huevo y está rodeado por una hilera de polígonos coriónicos. En *A. serpentina* el micrópilo se localiza en una prominencia muy cerca del ápice del huevo. Normalmente los aerópilos se encuentran en el extremo anterior, lado ventral, de los huevos. Los huevos fracturados de ambas especies mostraron que el corion es cavernoso a lo largo del cuerpo del huevo. Se sugiere que estos huevos pueden realizar la respiración por medio de un plastrón. Los resultados sugieren que la morfología del huevo puede ser útil para elucidar las relaciones taxonómicas y filogenéticas entre las especies de *Anastrepha*.

The genus *Anastrepha* comprises about 180 species which occur in South and Central America, in the West Indies, and with few species being found in southern USA (White & Elson-Harris 1992). Although numerous works have been focused on several biological parameters of these flies, description of the eggs were made for only a few species, despite the fact that egg morphology may have taxonomic applications (Norrbom 1985).

Similar to eggs of other Tephritidae and Diptera Cyclorrhapha (see Ferrar 1987), the eggs of *Anastrepha* species may present a sculptured or smooth chorion. In the

former class are included *A. fraterculus*, *A. bistrigata*, *A. striata*, *A. suspensa*, *A. obliqua*, *A. ludens* (Emmart 1933, Sein 1933, Lawrence 1979, Norrbom 1985, Steck & Malavasi 1988, Carroll & Wharton 1989, Murillo & Jirón 1994, Selivon & Perondini 1998), although variations in the two last ones were detected (Norrbom 1985, Carroll & Warthon 1989, Norrbom & Foote 1989, Murillo & Jirón 1994). On the other hand, the eggs of *A. serpentina*, *A. cordata*, *A. grandis*, *A. leptozona* and *A. pittieri*, present a chorion without decoration (Emmart 1933, Norrbom 1985, Steck & Wharton 1988). Another morphological feature is the presence of respiratory horns or appendages in some species, such as *A. obliqua* (Emmart 1933, Norrbom 1985, Murillo & Jirón 1994), *A. pittieri* and *A. nigrifascia* (Norrbom 1985).

Herein, we describe the morphology of the eggs of *Anastrepha sororcula* Zucchi and present also a redescription by scanning electron microscopy of *A. serpentina* (Wiedemann) eggs, previously studied by Emmart (1933).

Materials and Methods

The laboratory strain of *Anastrepha sororcula* Zucchi used in the present work, derived from infested guava fruits (*Psidium guajava*) which were cultivated in Conceição do Almeida (12°30'S, 39°10'W), the state of Bahia. The strain of *A. serpentina* (Wied.) was initiated with flies derived from "abricó" fruits (*Manilkara zapotilla)* collected in São Sebastião (23°40'S, 45°20'W), the state of São Paulo. The flies were fed water and a 3:1 mixture of sugar and corn protein hydrolysate. For oviposition, guavas and papayas (*Carica papaya)* were furnished, respectively for *A. sororcula* and *A. serpentina*.

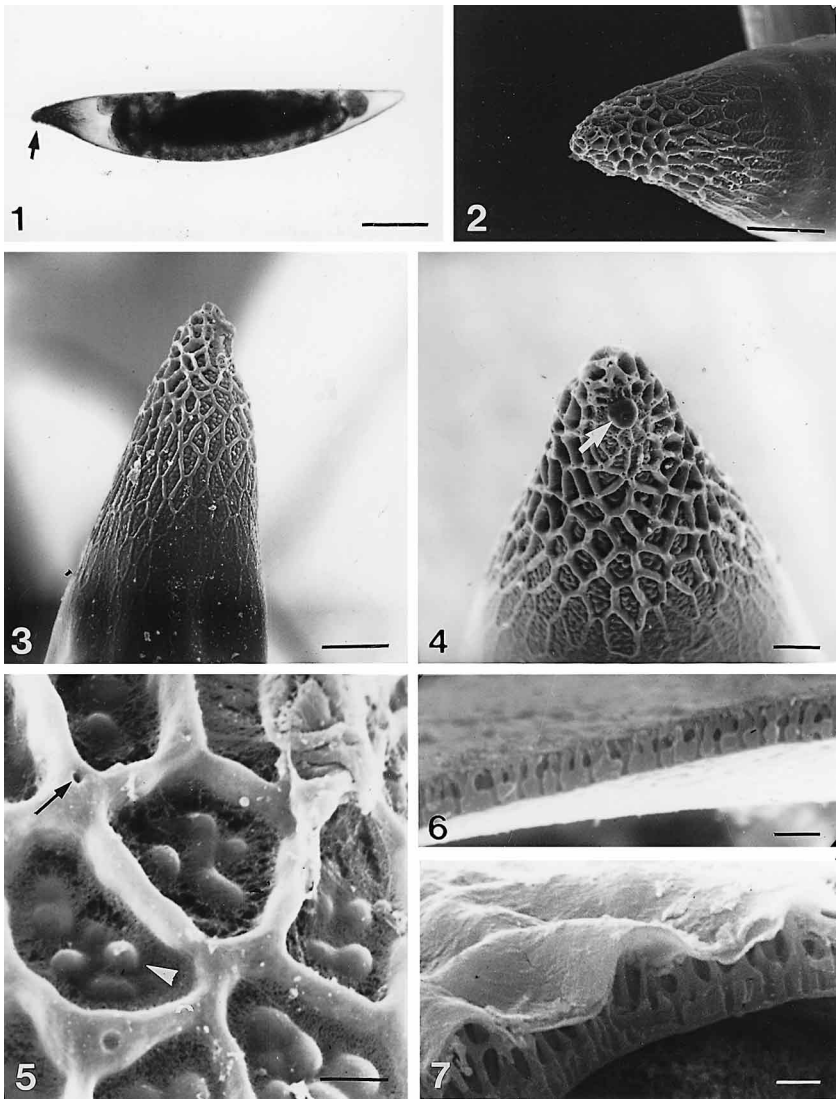
For the collection of *A. sororcula* eggs, the guavas were replaced by artificial substrates, which consisted of 2% agar hemispheres, stained with red aniline and wrapped in Parafilm® "M" (mod. Boller 1968). The eggs of *A. serpentina* were obtained directly from the papayas. The eggs were transferred to depression slides and immersed in fixative (2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4), post fixed in 1% osmium tetraoxide and dehydrated in an alcohol series. After critical point drying and "sputtering", the gold covered specimens were examined in a Zeiss DMS 940 scanning electron microscope.

Results

Anastrepha sororcula

The eggs of *A. sororcula* (n = 20) have an average length of 1.11 ± 0.12 mm and a mean diameter (in the broadest region) of 0.19 ± 0.01 mm. They are whitish, slightly curved, the curvature of the ventral side being more pronounced than the dorsal one, which is almost straight. The egg is broader from the middle toward the anterior third and tapers gradually toward the posterior pole (Fig. 1). At the anterior end the ventral side curves more abruptly than the dorsal, causing the egg apex to be asymmetric. The apex is formed by a pointed papilla, which bends to the ventral side (Figs. 1, 2). In cross section the egg is symmetrical and almost circular.

The chorion presents a well-developed lattice of ridges in a polygonal arrangement covering the anterior pole of the egg (Figs. 2, 3, 5). The sculpturing fades posteriorly and no ornamentation is found elsewhere on the eggs. Starting from the apex and around the egg circumference, the lattice is made of several tiers of polygons of different sizes but showing a regular shape, that is, in each one the sides are of similar length (Figs. 2, 3). However, the regular polygons are followed by several rows of elon-



Figs. 1-7. *Anastrepha sororcula* eggs: 1, light microscope view of an egg submerged in water. The arrow points to the papilla at the anterior end; 2-5, Scanning electron microscope (SEM) views of the anterior end of eggs: 2,shows that the ornamentation covers a larger area in the ventral side (at bottom), 3, the polygons ridges are more developed on ventral (left) than the dorsal side,4, the micropyle at the dorsal side near the apex (arrow), 5, enlargement of an area at the anterior end showing the polygons, the protuberances (arrowhead) and aeropyles (arrow); 6, 7, fractured eggs showing the internal structure of the chorion at a median (6) and anterior region (7) of the egg with the folds of the polygon ridges. Scale bars in (1) = 200 μm; (2,3) = 50 μm; (4) = 20 μm; (5) = 5 μm; (6, 7) = 2 μm.

gated ones, before they disappear. The sculpturing is asymmetric, the polygon ridges on the ventral side being more developed than those in the dorsal of the eggs (Fig. 2).

The micropyle is located at the dorsal side of the papilla and there is a row of small chorionic polygons around it, as shown in Fig. 4. Circular openings, the aeropyles, occur near the papilla. These orifices are usually found in the corners of the polygons were two or three ridges meet (Figs. 2, 4), and around the egg circumference.

The chorion in the internal areas of the polygons, present an irregular surface with round protuberances (Figs. 3, 5). These protuberances are fused together in the polygons more distal from the apex. However, in the proximal ones, the protuberances seem to be detached from each other and from the walls of the polygons. They are connected through a network of fibrilles (Fig. 5).

The morphological features above described were observed in a sample of 15 eggs, and conspicuous differences were not detected.

The chorion is cavernous exhibiting a continuous tunneling of open spaces over the entire egg body. This can be seen in a fractured egg, as shown in Fig. 6. Internally, the chorion seems to be made of a basal layer traversed by channels, an intermediate region of pillars and large open spaces, and another layer formed by branching of the pillars. On the external side, there is a fine layer of irregular appearance covering the egg surface.

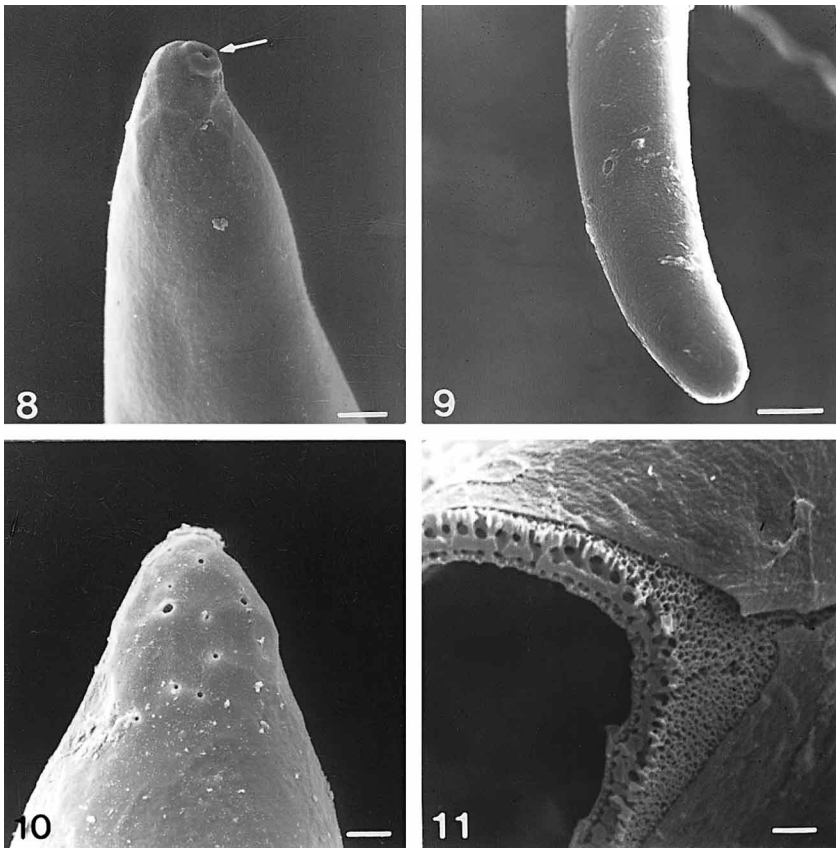
Anastrepha serpentina

The eggs of *A. serpentina* (n = 20) show an average length of 1.66 ± 0.08 mm and a mean broadest diameter of 0.21 ± 0.01 mm. They are creamy white, with a anterior end and a slender tapering toward the posterior pole. The eggs are curved, the convex side being ventral. The micropyle is located almost at the anterior tip and is surrounded by a salient rim (Fig. 8). The entire egg surface is smooth chorion without sculpturing (Figs. 8, 9). These characteristics are in accordance with the description of the eggs of this species made by Emmart (1933). The present study by SEM also shows that at the anterior end polygonal marks are found, although barely visible (Figs. 8, 10). A number of aeropyles occur in a region at the ventral surface near the anterior pole. These orifices were never observed in the dorsal side of the eggs (Fig. 10). No conspicuous morphological differences were found in a sample of 15 eggs.

In fractured eggs, five layers could be distinguished forming the internal structure of the chorion (Fig. 11). The most internal one, is thin and makes projections toward the surface which expand considerably in the median region forming a thick intermediate layer. From this layer up, new projections are found branching near the egg surface. In the two regions occupied by the projections, open spaces are found. These two areas of open spaces communicates with each other by tunnels that cross the intermediate layer of dense material. These open spaces are also continuous to the open areas among the branches of dense material nearest to the egg surface. These outermost branches fuse to each other actually forming a perforated layer. On the outside, there is a layer of uniform consistency covering the egg surface (Fig. 11).

DISCUSSION

The eggshell in *A. sororcula* is, in several aspects, similar to those found in other species of the genus by presenting an area of sculpturing of the chorion covering a region around the micropylar end of the eggs. On the other hand, they differ from the eggs of other fruit flies in which no decoration of the chorion is present, such as *A. serpentina*, described earlier by Emmart (1933) and restudied in the present work. These



Figs. 8-11. SEM views of *Anastrepha serpentina* eggs. 8, side view of anterior end showing the micropyle (arrow) at the dorsal side; 9, posterior end without ornamentation; 10, ventral view of anterior end with aeropyles; 11, fractured chorion showing the complex structure of pillars and open spaces. Scale bars in (8) = 20 μm; (9) = 50 μm; (10) = 10 μm; (11) = 2 μm.

two general classes of eggs, are found not only in genus *Anastrepha,* but also in other frugivorous (Persson 1963, Margaritis 1985) and non-frugivorous Tephritidae species (e.g., Haseler 1965, Novak & Foote 1968, Headrick & Goeden 1990), being, actually, a common feature of insect eggs (see Hinton, 1969, 1981; Ferrar, 1987).

Another feature of the eggs of both species here analysed, is the absence of a conspicuous respiratory appendage on the anterior end, like those found in other frugivorous fruit flies, such as *A. obliqua* (Emmart 1933, Norrbom 1985, Murillo & Jirón 1994), *A. nigrifascia* and *A. pittieri* (Norrbom 1985) and in non-frugivorous species, such as *Paracantha gentilis* (Headrick & Goeden 1990) among others. However, the sculpturing, the internal tunneling and the aeropyles probably represent morphological adaptations of the chorion to provide atmospheric air to the developing embryos, as is known to occur in eggs of other insect species (Hinton 1969, 1981). The open in-

ternal spaces may hold a layer of gas that extends throughout the egg body. These adaptations could provide the eggs of fruit flies with the capacity for a plastron-mediated respiration, if they are submerged in water. This situation might be met by these eggs inside the fruit tissues. Even for the eggs of *A. serpentina*, in which no sculpturing is found, the presence of aeropyles and the open spaces within the chorion could also provide conditions for a plastron respiration. However, these suggestions must await further studies since no experimental demonstration of plastron respiration in Tephritidae eggs is found in the literature. The internal structure of the chorion of *A. sororcula* and *A. serpentina* eggs seems to present a complexity similar to that of other *Anastrepha* species (Murillo & Jirón 1994, Selivon & Perondini 1998), and of *Ceratitis capitata* (Margaritis 1985) by showing several layers and open spaces, but is different from the chorion of *Bactrocera (Dacus) oleae* (Margaritis 1985), and *Rhagoletis cerasi* (Mouzaki & Margaritis 1991) eggs which show less complexity of layers.

The morphological similarities of *A. sororcula* eggs to those of the other species of the "*fraterculus* group" so far studied, *A. fraterculus, A. ludens, A. obliqua* and *A. suspensa*, reflect the close taxonomic relationships among these species showed not only by morphological characteristics (Norrbom & Kim 1988, White & Elson-Harris 1992) but also by genetic data (Morgante et al. 1980, Selivon 1996). Conversely, its distance to *A. serpentina* is corroborated by the same criteria. Thus far, egg morphology seems to be a character that may indeed be useful to elucidate taxonomic relationships among fruit flies, as suggested by Norrbom (1985).

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MIDGE RESISTANCE AND HYDROCYANIC ACID CONTENT OF *SORGHUM BICOLOR*

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Sorghum midge (*Stenodiplosis sorghicola* [Coquillett]) is found in most countries where sorghum (*Sorghum bicolor* L. Moench) is grown (Doggett 1988). A number of sorghum lines resistant to the sorghum midge have been identified (Teetes 1985). Various mechanisms have been proposed for midge resistance, including cleistogamy, nonpreference, antibiosis, tannin content, and glume size and tightness (Doggett 1988 and Teetes 1985).

Dhurrin[p-hydroxy-(S)-mandelonitrile-$-D-glucoside] is the cyanogen of sorghum (Gorz et al. 1977) that is enzymatically hydrolyzed to cyanide and p-hydroxybenzaldehyde (p-HB) when cellular integrity is interrupted (Wajant et al. 1994). Gorz et al. (1977) reviewed studies that showed colorimetric methods can be used to determine cyanogen content or hydrocyanic acid potential (HCN-p) in plant tissue.

In an unrelated study in search for cyclic hydroxamic acids (e.g., DIMBOA), the second author (M. D. Richardson) found that midge resistant sorghum genotypes, Huerin and Tift MR88, produced 0.52 and 0.78 mg p-HB while midge intermediate (genotype 1821 cm) and midge susceptible (Tx623B) genotypes produced 0.18 and 0.15 mg p-HB per g fresh weight [LSD(0.05) = 0.10], respectively (unpublished data). The objective of this study was to determine whether resistance to sorghum midge may be related to HCN levels.

In this study, three replications (pots) of 10 genotypes (Table 1) of sorghum with varying levels of midge resistance were planted in 20 cm wide plastic pots in the greenhouse on 17 Nov 1993 in a randomized complete block design. Plants were thinned to six plants per pot at ten days after planting. Response of the genotypes to midge (Table 1) were determined in a previous unpublished study, where a rating of 0 = resistant and 9 = susceptible. These entries were part of a larger experiment arranged in a randomized complete block with four replications where genotypes were rated for midge resistance under natural midge infestation at Tifton, Georgia. All genotypes in this study were inbred lines except DeKalb E57, Hyperformer, AgraTech 712G and Pioneer 8333 which were F_1 hybrids. Plants were grown under uniform temperature (28 to 30E C) and moisture conditions in the greenhouse. Plants were fertilized with a complete water-soluble fertilizer to maintain healthy growth. HCN contents were analyzed by analysis of variance procedures (SAS Institute, Inc., 1985) to evaluate genotype effects. Differences between genotype means were separated by the least significance difference (LSD) test at the 0.05 probability level.

Leaf discs from non-expanded leaves of the six plants (50 days old) in a pot were cut with a #3 cork cutter and placed in 10 ml test tubes. Leaf tissue from each genotype in each replication weighed 0.07 g. No significant differences in dry weights of the leaf tissue were observed among replications and genotypes. Four drops of chloroform were added to each test tube. We used the colorimetric technique and standards described by Hogg and Ahlgren (1942). HCN content of the solutions were determined using a Milton Roy Company Spectronic 501 at 540 nm with Mr. Sipper.

TABLE 1. HYDROCYANIC ACID (HCN) CONTENT OF SORGHUM GENOTYPES WITH VARYING LEVELS OF MIDGE RESISTANCE.

	Sorghum	
Genotype	Response to midge[†]	HCN content (mg/g fresh weight)[‡]
DeKalb E57	9.0 a	0.11 ± 0.05 e
Pioneer 8333	8.3 a	0.19 ± 0.01 d
AgraTech 712G	8.0 a	0.19 ± 0.02 d
1821 cm	6.0 b	0.23 ± 0.05 cd
Hyperformer	5.8 b	0.19 ± 0.01 d
Tift 9110	3.3 c	0.26 ± 0.01 cd
Huerin	3.0 cd	0.39 ± 0.04 a
TAM 2566	2.5 cd	0.34 ± 0.04 ab
Tift MR88	2.0 d	0.26 ± 0.01 cd
PI383856 (AF28)	0.3 e	0.29 ± 0.01 bc

[†]Ratings from unrelated-unpublished study where 0 = resistant and 9 = susceptible.
[‡]Mean ± SE.
Means in each column followed by same letter are not different at P = 0.05.

The sorghum genotypes most resistant (with rating of 3.3 or lower) to the sorghum midge had numerically higher HCN content than the more susceptible (with ratings of 5.8 or above) genotypes (Table 1). The most midge-susceptible genotype, DeKalb E57, had significantly (P= 0.05) lower HCN content than the remainder of the genotypes. Midge-resistant genotypes TAM 2566 and Huerin had significantly higher levels of HCN than all of the more susceptible (rating of 5.8 or higher) genotypes. The HCN content of midge-resistant genotypes Tift MR88 and Tift 9110 was numerically higher but not significantly different from susceptible genotypes Hyperformer, AgraTech 712G, Pioneer 8333 or 1821 cm. It appears that HCN may be related to resistance in sorghum to the sorghum midge and that this resistance may be imparted at a certain threshold level of HCN. It may be argued that this study measured HCN in the leaves which may not relate to HCN levels in the panicle. We could not determine the HCN-p in the panicles of the test genotypes at the same time in this study because of the broad range in days to flowering among the genotypes. We did test the HCN level in the florets of 1821 cm and found it to be 0. 21 mg HCN per gram fresh weight which is slightly less than observed in the leaf tissue (Table 1).

It is known that cyanogenic compounds enzymatically release cyanide as a cell is damaged by a factor such as a feeding insect (Wajant et al. 1994). This localized release of cyanide at an injury site could produce the chemical barrier necessary to prevent further damage by the insect. Cyanogenic compounds are but one example of the many secondary plant metabolites that protect plants against various biotic pests. Further work is needed to determine the relationship of HCN and/or p-hydroxybenzaldehyde in sorghum panicles to midge resistance and to assess the variability for these compounds among a wide range of sorghum germplasms. The authors acknowledge the laboratory assistance of Jacolyn Merriman and Ellen Tucker.

SUMMARY

Two sorghum genotypes highly resistant to sorghum midge had significantly higher levels of hydrocyanic acid than a highly susceptible genotype. It appeared that sorghum midge resistance in sorghum may be related to a threshold level of hydrocyanic acid.

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AN ALARM PHEROMONE FROM HEADS OF WORKER *VESPULA SQUAMOSA* (HYMENOPTERA: VESPIDAE).

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Several species of yellowjacket wasps (*Vespula* and *Dolichovespula* spp.) possess alarm pheromones associated with the venom that elicit recruitment, attraction, and stinging attacks if a suitable visual target is provided. Maschwitz (1964a & b) demonstrated alarm responses in workers of the common yellowjacket *Vespula vulgaris* L. and in the German wasp *Vespula germanica* (F.) in response to squashed venom sacs and solvent extracts of conspecific venom sacs, identifying the venom as the source of an alarm stimulus. His findings were confirmed by Aldiss (1983). Alarm responses to extracts of the venom sac and associated glands have also been observed for *Dolichovespula saxonica* (F.) (Maschwitz 1984), the southern yellowjacket *Vespula squamosa* (Drury) (Landolt and Heath 1987) and the eastern yellowjacket *Vespula maculifrons* (Buysson) (Landolt et al. 1995). The chemical N-3-methylbutylacetamide was identi-

fied as an alarm pheromone from the venom of the southern and eastern yellowjackets (Heath and Landolt 1988, Landolt et al. 1995).

There is some evidence that an additional alarm pheromone may be produced by yellowjackets, in glands associated with the head. Yellowjacket workers possess up to eight sets of exocrine glands in the head (Landolt and Akre 1979), but little is known of the functions or chemistry of their exocrine secretions (Downing 1991, Jeanne 1993). Aldiss (1983) demonstrated significant numbers of hits to a target by worker *V. vulgaris* in response to crushed heads of conspecific workers applied to cotton dental rolls. However, Maschwitz (1964b, 1984) did not observe an alarm response by workers of *V. vulgaris* or *D. saxonica* to crushed heads of conspecific workers, and Landolt and Heath (1987) found no response by southern yellowjackets to a methylene chloride extract of heads of conspecific workers. These disparities may be due to differences in experimental methods, including assay criteria and methods of preparing and presenting stimuli.

We hypothesize that an alarm pheromone from the head is used by the southern yellowjacket, in addition to the venom alarm pheromone N-3-methylbutylacetamide (Heath and Landolt 1988). In previous experiments with this species (Landolt and Heath 1987), a distinctive odor was evident from wasps vacuumed from nests and from objects and clothing that had been attacked by wasps. This odor is not similar to that of the alarm pheromone N-3-methylbutylacetamide and seems to be most potent in the head of the southern yellowjacket worker. Also, observations of southern yellowjackets attacking small corks presented near their nest entrances indicated that these wasps bite and chew in addition to stinging, when they attack. We report here alarm and attack responses from southern yellowjackets to extracts of conspecific heads, indicating a second alarm pheromone in this species originating from the head.

All studies were conducted in Alachua County and Sarasota County, Florida, with vigorous underground colonies of the southern yellowjacket. Workers were collected from active colonies using a shop vacuum with an attached trap. Wasps collected were stored for several weeks in a freezer at -60°C until extracted. Heads of 200 wasps were severed with a razor blade and placed in 4 ml of methanol in a pestle and were ground into a paste. The supernatant was pipetted into a 5 ml glass vial and was subsequently brought back up to a 4 ml volume with methanol. The treatment protocol was the application of 100 microliters of this solution (5 wasp equivalents) to a 5.5 cm diam filter paper attached to a 15 cm diam black sphere coated with tanglefoot and attached to a 0.5 m long wire. After the evaporation of the solvent (ca 30 sec.) from the filter paper, the sphere was moved to a one m distance from a wasp colony entrance, with the wire implanted into soil. Numbers of attacking wasps captured on a sphere were counted after a period of 2 min. As a control, 100 microliters of methanol was applied to a filter paper on an identical sphere presented in the same manner prior to each treatment replicate. This assay was conducted 10 times, using 4 different colonies of the southern yellowjacket. It was noted that the head extract used for this assay and the treated filter papers did possess the distinctive odor referred to above.

The pooled methanol extract of yellowjacket heads was analyzed by GC-MS for the presence of N-3-methylbutylacetamide using the methods of Heath and Landolt (1988). This analysis was conducted to determine if worker heads may have been contaminated with alarm pheromone from venom during wasp collection.

Significant numbers of southern yellowjackets were attracted and contacted the spheres in response to the head extract ($t = 2.35$, $p < 0.05$, $df = 9$) (Table 1). Numbers of wasps captured on the black 15 cm diam sphere coated with tanglefoot ranged from 0 to 224 when baited with a 5 wasp equivalent dose of methanol extract of conspecific heads, while 0 to 2 were captured on control spheres baited with methanol alone. N-3-methylbutylacetamide was not detected in the methanol extract of heads, indicating

that the alarm pheromone activity of those extracts was due to an additional pheromone. Estimated limit of detection was 0.2 ng per wasp equivalent of head extract.

Our results with the southern yellowjacket provide the second account (see Aldiss 1983) of alarm activity in a vespid originating from heads. Aldiss (1983) elicited alarm responses in the common yellowjacket with crushed conspecific heads applied to cotton dental wicks. Like the southern yellowjacket, the common yellowjacket also possesses an alarm pheromone in the venom. The failure of previous studies with the southern yellowjacket to obtain responses to extracts of heads (Landolt and Heath 1987) may have been due to the solvent used in the extractions. Previous studies were conducted using methylene chloride (Landolt and Heath 1987) whereas we used methanol in this study. Methanol was chosen after determining that the distinctive odor present in southern yellowjackets and evident following their attacks on corks, clothing, and assay spheres, was not readily extractable with hexane or methylene chloride, but was extracted with methanol. However, it is not known if this odor, similar to butterscotch to the human nose, is part of the alarm pheromone from the heads of southern yellowjackets.

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SUMMARY

Workers of the southern yellowjacket, *Vespula squamos* (Drury) responded to methanolic extracts of conspecific worker heads with attraction and attack, evidenced by significant numbers captured on treated black spheres presented near nest entrances. This species appears to have alarm pheromones that originate both in the venom and in glands in the head. The venom pheromone N-3-methylbutylacetamide

TABLE 1. NUMBERS OF WORKER SOUTHERN YELLOWJACKETS ATTACKING A TREATED 15 CM DIAM BLACK SPHERE TREATED WITH METHANOL AS A CONTROL OR WITH A 5-WASP EQUIVALENT DOSE OF A METHANOL EXTRACT OF CONSPECIFIC WORKER HEADS (2 MIN ASSAY). ALACHUA AND SARASOTA COUNTIES, FLORIDA, 1992-1994.

		Numbers of "Hits"		
Rep.	Date	Control	Head Extract	Colony
1	14 Dec. 1992	0	24	A
2	15 Dec. 1992	0	108	A
3	21 Dec. 1992	0	0	B
4.	21 Dec. 1992	0	1	B
5	21 Dec. 1992	0	0	B
6	21 Dec. 1992	0	3	B
7.	21 Dec. 1992	1	66	B
8.	21 Dec. 1992	1	24	B
9.	4 Nov. 1993	0	224	C
10	17 Mar 1994	2	86	D
$\bar{x}$ ± SE		0.4 ± 0.2	53.6 ± 22.6	

was not detected in GC-MS analyses of *Vespula squamosa* head extracts. Behavior of attacking wasps suggests possible alarm pheromone application by the mandibles.

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ARGYRODES IN WEBS OF THE FLORIDIAN RED WIDOW SPIDER (ARANEAE: THERIDIIDAE)

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Spiders of the genus *Argyrodes* Simon 1864 (family Theridiidae, cobweb spiders) live almost exclusively in the webs of other spiders. *Argyrodes* species may interact with their hosts in several ways, as a kleptoparasite stealing the host's prey, as a host predator, web-stealer or commensal (see Cangialosi 1997 for discussion). Many *Argyrodes* species occur in the tropics and subtropics. They are often found in webs of var-

ious species of the genera *Argiope* Audouin 1826 (Araneidae), *Nephila* Leach 1815 (Tetragnathidae), *Agelenopsis* Giebel 1869 (Agelenidae), *Neriene* Blackwall 1833 (Linyphiidae) and *Achaearanea* Strand 1929 (Theridiidae; see Exline & Levi 1962).

In the course of behavioral studies on a Floridian endemic spider, the red widow *Latrodectus bishopi* Kaston 1938 (Theridiidae; Marion County, Ocala National Forest, along Hwy 40, 1/4 mile west of Central Lookout Tower) three different species of *Argyrodes* were collected from their hosts' webs. *Latrodectus bishopi*, limited to Central and South Florida (Kaston 1970; Levi & Levi 1990), builds its web on palmetto shrubs (Genus *Sabal*) in oak scrub-sand pine woods. The base of the web consists of a large, dense, slightly convex sheet, with an extensive three-dimensional large-meshed network of threads above the sheet and a densely woven, funnel-shaped retreat attached to the convex sheet. The retreat is always placed in an unopened palmetto leaf, with the opening of the retreat funnel directed upwards. During the six-week observation period (July and August) the red widow spiders spent most of their daytime hours in the retreat. Daily, 51 websites of young, subadult and adult female and male *L. bishopi* webs were monitored. All *Argyrodes* specimens were found only on webs of adult or subadult female *L. bishopi*, never in the webs of males or very young widow specimens. *Argyrodes* was always found in the large-meshed network above the convex sheet, never in the retreat or the convex sheet. The following three species of *Argyrodes* were found in *L. bishopi* webs at the above mentioned location: *Argyrodes elevatus* Taczanowski 1872; *Argyrodes furcatus* (O. P.-Cambridge 1898); *Argyrodes caudatus* (Taczanowski 1873), see Table 1. The *Argyrodes* species composition on individual *L. bishopi* webs was not recorded.

On August 12 and 13 all 51 *L. bishopi* websites were checked for *Argyrodes* specimens. On August 12, fifteen webs were found to carry *Argyrodes* specimens and all *Argyrodes* specimens were collected. Twenty-four hours later, the same *L. bishopi* webs were monitored again; eleven of the fifteen webs had *Argyrodes* specimens. Within 24 hours, the population of *A. furcatus* was restored to 50% of the original number. Possible *Argyrodes* recruitment sites (webs of other potential hosts) were not investigated. Distances between owner-occupied *L. bishopi* webs ranged from 2.1m to 10.8m; distances between *Argyrodes*-invaded widow webs ranged from 2.1m to 8.4m. The observations in the present note suggest that webs of other spider species living in close proximity to *L. bishopi* webs may also harbor *Argyrodes* specimens and that movements between these different host webs may occur.

On 26 July 1997 an adult female of *A. furcatus* was collected while feeding on a dead juvenile *L. bishopi*. Whether *A. furcatus* had caught the widow host or was just feeding on a dead host spider could not be determined. During the six-week observa-

TABLE 1. COLLECTION DATES, NUMBER OF COLLECTED *ARGYRODES* SPECIMENS AND GENDER DISTRIBUTION.

Date	A. elevatus	*A. furcatus*	A. caudatus
17 Jul 1997	1♂, 2♀	4♂, 3♀	2♂, 2♀
31 Jul 1997	—	4♂, 28♀	1♂, 1♂juv, 2♀
12 Aug 1997[1]	1♂, 1♀	13♂, 30♀	3♂, 1♂juv, 3♀
13 Aug 1997[2]	—	6♂, 15♀	1♂, 1♀

[1]Collected in 15 *L. bishopi* webs.
[2]Collected in 11 *L. bishopi* webs.

tion period, prey capture and courtship behavior between *Argyrodes* males and females were observed frequently. *Argyrodes* specimens were found to reside in empty *L. bishopi* webs, but they were never observed in host-free webs built by a different species than *L. bishopi* on the palmetto shrubs.

Egg sacs of *A. furcatus* were collected with adult females and are described here for the first time. Single light-brown spindle-shaped egg sacs hang from thick silk threads in the three-dimensional network of the widow web. The egg sac ends with a round silk collar, and is very similar to the egg sac of western *A. baboquivari* as figured by Exline & Levi (1962: fig. 2).

Several aspects of the relationship between *Argyrodes* and its hosts, e.g., host-specificity, territoriality among various *Argyrodes* species, movement of *Argyrodes* invaders among host webs of the same or of different species and others can be investigated conveniently at the described location. *Argyrodes* and *Latrodectus* voucher specimens are deposited at the Field Museum.

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Summary

Specimens of *Argyrodes elevatus, Argyrodes furcatus,* and *Argyrodes caudatus* were found in the three-dimensional large-meshed network above the convex sheet in large webs of subadult and adult females of the Floridian red widow, *Latrodectus bishopi*. The egg sac of *A. furcatus* is described for the first time. *Argyrodes* specimens were also observed eating their host and remaining in empty host webs during the six-week observation period.

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OBSERVATIONS ON THE OVIPOSITION PROCESS OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

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A single adult *Diaprepes abbreviatus* L. was discovered in a citrus nursery in Orange County Florida (Woodruff 1964). *D. abbreviatus* was not collected again until 1968, when larvae were collected at the same nursery and several hundred adults and larvae were subsequently collected in and around Apopka, FL (Woodruff 1968). Since its introduction in 1964, *D. abbreviatus* has spread to 20 counties in Florida, where it currently infests approximately 164,000 acres (66,420 ha) (Anonymous 1997). This area contains approximately 30,000 acres (12,150 ha) of infested commercial citrus and has a limited and localized geographical distribution.

D. abbreviatus is an insidious pest; tree symptoms may not appear until the larvae are well established on the tree roots (Wolcott, 1936). Adults feed on young foliage, females lay egg clusters between leaves, and the larvae feed on the roots of a host (Fennah 1942, Jones 1915, Wolcott 1936, Woodruff 1968). Oviposition by *D. abbreviatus* appears to be restricted to nighttime hours (Jones 1915, Schroeder 1981, Wolcott 1936). Females oviposit approximately 60 egg masses during their lifetime which may contain from 30 to 260 eggs each and an average of about 5,000 eggs over their life span (Wolcott 1936). Wolcott (1933) observed that female *D. abbreviatus* preferred to oviposit between paper strips compared to leaves. Neonate larvae failed to emerge from these strips (Wolcott 1933). Fennah (1942) found that *D. abbreviatus* preferred paper strips over tin foil strips and mature leaves which were both favored over immature leaves for oviposition. Immature leaves are preferred feeding sites (Jones 1915, Wolcott 1936). Adair et al. (1998) reported that freezer paper strips might serve as a potential oviposition trap in citrus.

Adult *D. abbreviatus* were field collected in Vero Beach, Florida and maintained as previously described (Adair et al. 1998). Thirty females and 30 males per cage were held in 30 × 30 × 60 cm aluminum rearing cages (Bioquip Products, Gardena, CA 90248-3602) at 27 ± 2°C, 30% RH and photoperiod of 11:13 (L:D). Five 2.54 cm × 15.24 cm doubled strips of transparency film (polyester, Labelon, Canadaigua, NY 14424) were provided for oviposition sites. At 10:00 PM, transparency film strips containing an ovipositing female were removed to a microscope stage for observation and photographing.

Microscopic observations were conducted with an Olympus S2-6045 zoom stereo microscope with a 100AL 0.5× objective lens, 10× eyepiece and a NFK 2.5 × LD 125 lens and Olympus S2-PT (with L-adapter) adapter for a SLR camera. An Olympus OM 2S camera with automatic shutter speed and f-stop was used with Kodak Kodacolor 400 Gold® film. The transparency film strip with an ovipositing female was placed across an open Petri dish and illuminated above and below with a tungsten halogen lamp (Olympus Highlight 3000) equipped with bifurcating cold fiber optic goose neck

illuminators. Ovipositing females were videotaped with a Sony Color Video camera (CCD-IRIS) top-mounted on the dissecting scope using standard recording mode to record time sequence data. Times were compiled for the following stages:

Ovipositor Extension (Fig. 1-1). Ovipositor moved down toward previously laid row of eggs. This process followed the resting stage. Ovipositor probed along row of eggs previously deposited to find position for next row. Rear legs were aligned in conjunction with ovipositor.

Lower Adhesive Deposition (Fig. 1-2). Timed from start of adhesive material deposition. Ovipositor was now at base of egg mass. Ovipositor moved in dabbing motion. Rear legs squeezed the transparency film thus spreading the adhesive.

Oviposition (Fig. 1-3, 1-4). A row of eggs was laid from bottom to top one by one. Each egg was placed approximately horizontal.

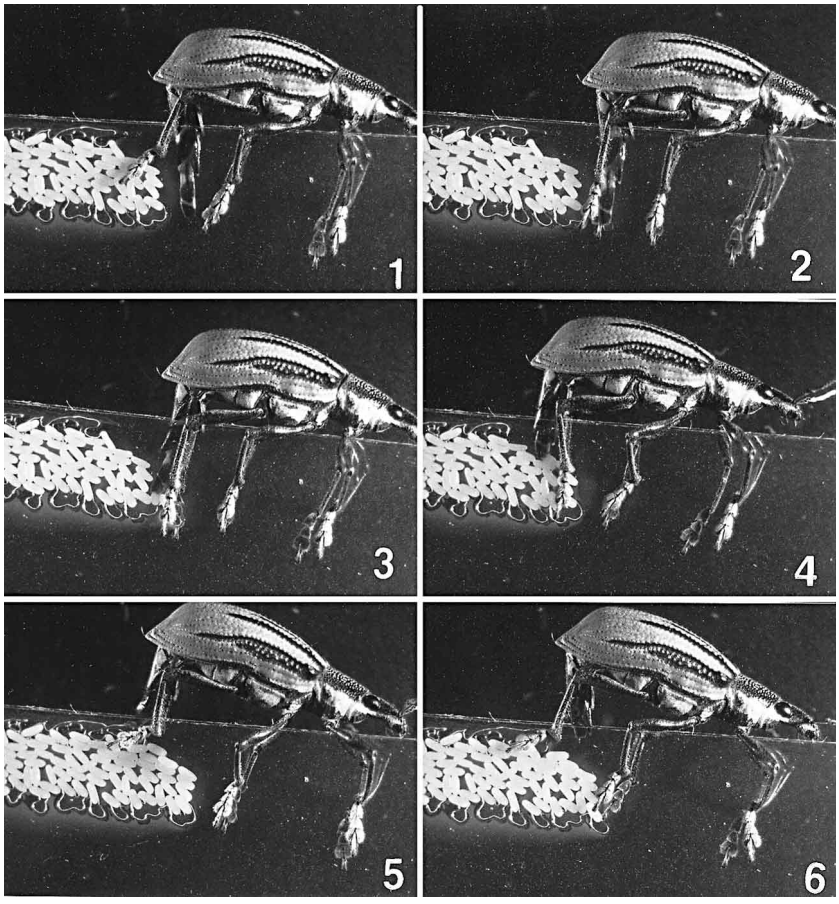

Fig. 1. Oviposition Postures of *Diaprepes abbreviatus*. 1. Ovipositor extension, 2. Deposition of lower egg mass adhesive, 3. Oviposition (lower), 4. Oviposition (upper), 5. Deposition of upper egg mass adhesive, and 6. Resting Posture.

Upper Adhesive Deposition (Fig. 1-5). Timed from start of adhesive deposition along upper surface of egg mass.

Rest (Figs. 1-6). Ovipositor withdrawn from egg mass to the edge of the transparency film and held at approximately 9% angle to horizontal edge of transparency film.

Twenty-one ovipositing females were observed and photographed. However, Fig. 1 is a sequence of photographs from one female. The female in Fig. 1 had laid approximately one-half of the egg mass when moved to the microscope stage. If a female was moved during the onset of oviposition or after the completion of oviposition, it dropped to the cage floor, typical evasive behavior of D. abbreviatus. When fully engaged in oviposition, females were easily moved to the microscope stage and completed production of an egg mass. Eight females were used for the stage time calculations. These females completed the 5 stages (above) 4 or more times (about 5.1 times per female). Forty-one replications were used for the time calculations.

Visual results are presented in the photographs of Fig. 1. The steps for oviposition and their time course (N = 41) in Fig. 1 are: ovipositor extension (30.8 ± 2.9 s mean ± S.D.), deposition of the lower egg mass adhesive (18.9 ± 1.9 s), oviposition (123.3 ± 12.0 s), deposition of the upper egg mass adhesive (22.7 ± 1.5), and rest (84.7 ± 10.7 s). This cycle averaged 4.7 min and was systematically repeated until all eggs in a single mass were deposited; 7-9 eggs were oviposited per cycle. Egg mass deposition time can be estimated by dividing the number of eggs in a mass by 8 and multiplying by 4.7 min (approximately 1.7 eggs/min). For example, a 100 egg mass would take approximately 1 h to deposit. Schroeder (1981) reported 1 h for an egg mass containing 80 + eggs; no data were presented.

After each egg was discharged females probed with the end of the ovipositor to position the next egg directly on top of the previous egg. Similarly, the ovipositor was used to locate the perimeter of the egg mass to deposit the upper and lower portions of the adhesive liquid. All females observed were consistent in applying the adhesive material only to the outer edges of the egg mass and none were observed depositing it inside the egg mass.

Summary

Oviposition by *D. abbreviatus* occurred in a six stage cycle of about 4.7 min. Consequently, a 100 egg egg mass would take about 1 h to complete. The six stages were documented photographically.

Endnote

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PARASITISM OF EASTERN LUBBER GRASSHOPPER BY *ANISIA SEROTINA* (DIPTERA: TACHINIDAE) IN FLORIDA

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The eastern lubber grasshopper, *Romalea microptera* Beauvois (= *guttata*; see Otte 1995) is a large romaleid grasshopper (adults = 2-12 g) that occurs sporadically throughout the southeastern USA, but in relatively high densities in the Everglades-Big Cypress area of south Florida (Rehn & Grant 1959, 1961). At this latitude, nymphs normally hatch from subterranean egg pods from January-April, eclose to adults from April-June, and oviposit from June-September. This grasshopper has been extensively studied due to its defensive attributes: they are gregarious, flightless, aposematically colored, expel an odorous secretion, and are toxic to birds and reptiles, but apparently not to most invertebrates (Jones et al. 1988, Whitman 1988, 1990, Whitman et al. 1991, 1992, Yosef & Whitman 1992, Hatle & Townsend 1996, Hatle & Spring 1998).

The New World tachinid genus *Anisia* (Subfamily: Goniinae; Tribe: Blondeliini) currently contains approximately 20 species (Wood 1985), which range from South America and the West Indies to southern Canada (Wulp 1890, Wood 1985). A few species are thought to parasitize Lepidoptera (Cole 1969, Arnaud 1978). However, *Anisia dampfi* (Aldrich 1927, Greene 1927) parasitizes various *Schistocerca* spp. grasshoppers in Central America (Greathead 1963, Arnaud 1978), *A. flaveola* (Coquillett) has been collected from a camel cricket in Florida (Chinn & Arnaud 1993), *A. gilvipes* (Coquillett) parasitizes crickets (Arnaud 1978), and *Tettigoniophaga vanini* Guimarães (?

= *Anisia)* (Wood 1985) appears to attack katydids. A closely related genus, *Phasmophaga*, apparently parasitizes walkingsticks (Patton 1958, Arnaud 1978, Wood 1985).

Very little is known about *Anisia serotina (Reinhard)*. The species was originally described as *Stenoneura serotina* by Reinhard (1945), from specimens collected in Texas, and was noted as such by Stone, et al. (1965). The species was transferred to the genus *Anisia* by Wood in 1985. As far as we know, these purely taxonomic works are the only published references to *A. serotina*.

During 10 years of studying *R. microptera* prior to 1992, we observed very low levels (usually 0-2%) of tachinid parasitization in lubber grasshoppers from Georgia and Florida. However, during 1992-1996, adult lubber densities in the Copeland-Ochopee area of south Florida were extremely high (> 900/100 m^2 at Copeland in 1994) and estimated tachinid parasitization rates in this area ranged from 2 to 10%.

In 1997, we observed that adult *R. microptera* densities in SW Florida were reduced (est. maximum density = 8/100 m^2 at some sites) and lubbers were heavily infested with maggots of *A. serotina*. Between 26-V-1997 and 10-VI-1997, we collected 950 *R. microptera* (20 nymphs and 930 adults) from Copeland and Oochopee, FL and brought them to Illinois State University. Evidence of parasitization was first noted when maggots literally dropped from field-collected grasshoppers. Within one hour of collecting, numerous mature maggots were observed in the bottom of the collecting buckets. Approximately 5% of the grasshoppers appeared weak or moribund at the time of collecting. Many also exuded a black tar-like substance from the anus, suggesting pathogenic or parasitoid infection. In addition, field-collected grasshoppers suffered a high mortality rate; within one month, 491 (51.7%) of the 950 grasshoppers died. This contrasted with previous years (1992-1996) in which tachinid infestation levels at Copeland, FL were <10% and grasshopper survivorship was high. Dissections of 45 of the dead grasshoppers (20 males, 20 females, and 5 juveniles) on June 11, 1997 revealed that 62.2% were parasitized with from 1 to 30 maggots ($\bar{x}$ = 9.18 maggots/infested grasshopper). Five of the parasitized grasshoppers were nymphs.

To determine infestation levels in living grasshoppers, we isolated 50 adult males and 50 adult females into individual 1-L containers with solid bottoms that trapped all emerging maggots. Lubbers were fed daily and maintained in the laboratory at 24-30°C. Grasshoppers were isolated on June 13, 1997 (approximately 3 days after the last removal of insects from the field) and maintained for 48 days, during which we recorded all emerging maggots. At the end of the isolation period, we dissected and examined all surviving grasshoppers for maggots (grasshoppers that died during the experiment were immediately dissected). We obtained the total parasitoid load for each lubber by summing the number of maggots that emerged and the number of maggots found during the dissection. The results (Table 1) show that significantly more females were infested (92%; 46/50) than males (72%; 36/50, X^2 = 6.78, df = 1, $p < 0.05$). The number of maggots per grasshopper ranged from 0 to 63 and averaged 7.0 ± 3.3 (SE) (n = 100). There was a strong trend, but no significant difference in the mean number of parasitoids in adult males ($\bar{x}$ = 5.1 ± 1.4 (SE), n = 50) vs. adult females ($\bar{x}$ = 8.9 ± 1.5 (SE), n = 50, t = 1.81, df = 98, $p > 0.05$). Similarly, when only parasitized grasshoppers were compared, there was no significant difference in the mean number of maggots in males ($\bar{x}$ = 7.1 + 1.8 (SE), n = 36) versus females ($\bar{x}$ = 9.7 ± 1.6 (SE), n = 46, t = 1.04, df = 80, $p > 0.05$).

In 1998, we returned twice to south Florida to survey lubber populations for tachinid infestation. We found both lubber and tachinid populations greatly reduced from previous years. Maximum adult lubber densities in the Copeland-Ochopee area were < 0.6/100 m^2 (approximately 1.0% of 1996 levels and 0.07% of 1994 levels).

Between 9-V-98 and 28-V-1998, we collected and dissected 52 lubbers (4 nymphs, 25 adult males, and 23 adult females) from an area extending from Everglades City

to Immokalee to Shark Valley, FL. We found only one maggot in a single adult male collected 15 miles N of Copeland, FL (Table 1).

Between 27-VII-1998 and 5-VIII-1998, we conducted a second survey and dissected 114 adult lubbers (84 males and 30 females) from an area extending from Copeland, to the Anhinga Trail, to Flamingo Key. Seven lubbers (3 females & 4 males) were infested with *A. serotina* maggots (Table 1). Three females contained 7, 22, and 35 maggots, whereas each of the four males contained a single maggot. Five of the parasitized lubbers were collected 10-14 miles N of Copeland, one male from Ochopee, and one male from the Anhinga Trail in the Everglades National Park. Summing all 1998 dissections across south Florida shows that 8/166 (4.8%) of dissected lubbers were infested with *A. serotina* maggots. The 1998 parasitization rate for just the Copeland-Ochopee area (Copeland, Ochopee, and 15 mi. N of Copeland) was 7.6% (7/92) (Table 1).

This is the first published host record for *A. serotina* and the first record of its occurrence in Florida. In addition, this is the first recorded parasitization in *R. microptera*. Indeed, neither Patton (1958), Rees (1973), Arnaud (1978), nor Fry (1987) mention either species. However, the US National Museum in Washington, D.C. contains *A. serotina* specimens reared from *R. microptera* in June 1966 from Hendry Co. and Lake Placid, FL (N. Woodley, pers. comm.).

We believe that our observed 1997 infestation rate (82%) is the highest ever observed for tachinids parasitizing grasshoppers. However, this probably represents a low estimate, because we did not isolate our grasshoppers until June 13, when many maggots may have already matured and emerged from their hosts. Our observations of 8.6 maggots per infested grasshopper with one male containing 63 maggots is also quite high, and surpasses Leonide's (1961) record of 62 tachinid (*Ceracia mucronifera* Rondani) maggots in a single *Anacridium aegyptium* L. grasshopper. Mature *A. serotina* maggots weigh ca. 0.038 g each. Hence, the mass of 63 mature *A. serotina* maggots is about 2.39 g or 72% of the mass of a mature Copeland-area male lubber (roughly 3.30g). Askew (1971) states that Tachinidae are almost without exception solitary endoparasitoids and it is unusual for more than one larva to survive in a single host. This is clearly not the case for tachinids attacking grasshoppers (Arnaud & Rentz 1965, Johnson et al. 1996).

This high level of parasitism is interesting because *R. microptera* is chemically defended and is unpalatable to many vertebrate predators (Jones et al. 1988, Whitman 1988, 1990, Whitman et al. 1991, 1992, Yosef & Whitman 1992). However, evidence suggests that some invertebrate predators are not deterred by lubber toxins (Whitman 1988, 1990). Indeed, many chemically defended arthropods are associated with specialized parasitoids or predators that have overcome their host's defenses (Reichstein et al. 1968, Askew 1971, Rothschild et al. 1973, Chapman & Page 1979, Eisner et al. 1980, Barbosa et al. 1986).

Our observations suggest an extremely dynamic host-parasitoid relationship between *R. microptera* and *A. serotina*. Although grasshopper-parasitoid populations often fluctuate widely in space and time (Prescott 1960, Greathead 1966, Farrow 1982, Chapman et al. 1986, Capinera 1987, Joern & Gaines 1990), our observed one-year (1997-1998) change in parasitization rate from 82% to 7.6% for the Copeland area is impressive. Just as striking, is the >99.9% reduction in lubber densities at Copeland, FL between 1994-1998. We do not know what drives these population fluctuations. Possibly, high lubber densities in the five years prior to 1997 may have allowed the tachinid population to build. Alternatively, weather may have contributed to the 1998 population crashes of both species. During 1997-98, south Florida experienced an unusually wet winter followed by a long spring drought, thought to have been influenced by an El Niño effect (NOAA 1997, 1998). Finally, alternative hosts may influence *Romalea-Ani-*

TABLE 1. PRESENCE OF *ANISIA SEROTINA* MAGGOTS IN LUBBER GRASSHOPPERS IN SOUTH FLORIDA IN 1997 AND 1998.

		n Dissected			n Parasitized			% Parasitized		
Date	Location	♂	♀	Total	♂	♀	Total	♂	♀	Total
VI-1997	Copeland-Ochopee	50	50	100	36	46	82	72	92	82
VI-1990	5 mi W Immokalee	3	2	5	0	0	0	0	0	0
	12-15 mi N copeland	11	4	15	1	0	1	9	0	7
	Copeland	1	4	5	0	0	0	0	0	0
	Ochopee	12	8	20	0	0	0	0	0	0
	16 mi W Shark Valley	1	3	4	0	0	0	0	0	0
	Shark Valley	0	3	3	0	0	0	0	0	0
VIII-1998	12-15 mi N Copeland	17	9	26	2	3	5	12	33	19
	Copeland	1	0	1	0	0	0	0	0	0
	Ochopee	20	5	25	1	0	1	5	0	4
	Shark Valley (EGNP)	14	8	22	0	0	0	0	0	0
	Anhinga Trail (EGNP)	18	3	21	1	0	1	6	0	5
	1-5 mi N Paurotis Pond (EGNP)	14	5	19	0	0	0	0	0	0
Total 1998		112	54	166	5	3	8	4	6	5

sia population dynamics. During our studies, *A. serotina* exhibited no diapause at room temperature (mature maggots pupated and eclosed in as few as 6 days). This suggests that *A. serotina* is multivoltine in south Florida, and may undergo numerous generations during a single lubber season (March-September). If *A. serotina* are active during the winter, when lubbers presumably are not available, they would need to utilize other hosts. Hence, tachinid densities in spring (when lubbers hatch) might depend on tachinid densities in winter, which in turn may depend on alternative hosts.

In any event, *Romalea-Anisia* interactions warrant further examination. *Anisia* may have potential as a biological control agent, not only for lubbers, which are minor garden and agricultural pests (Watson 1941, Griffiths & Thompson 1952), but also for more damaging Orthoptera.

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Summary

In 1997, 92% of female and 72% of male eastern lubber grasshoppers, *Romalea microptera Beauvois*, in southwest Florida were infested with maggots of the tachinid parasitoid *Anisia serotina* (Reinhard) (X = 8.6 maggots/infested grasshopper). In 1998, densities of both host and parasitoid were greatly reduced.

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THE RECOVERY AND APPARENT ESTABLISHMENT OF *CIRROSPILUS INGENUUS* (HYMENOPTERA: EULOPHIDAE) IN FLORIDA

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Cirrospilus ingenuus Gahan is an Asian parasitoid of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). Its natural range includes China, India, Indonesia, Japan, Malaysia, Taiwan and Thailand (Schauff et al. 1998). It has commonly been treated under the name *C. quadristriatus* (Subba Rao & Ramamani). However this name was recently synonymized with *C. ingenuus* by G. Evans (as a personal communication in Ujiye and Adachi, 1995:96).

Citrus leafminer invaded Florida in 1993 (Heppner 1993), and has been the subject of biological control attempts since that time (Hoy & Nguyen 1997). In 1994, *C. ingenuus* was released in limited numbers in Florida as a biological control agent of the citrus leafminer (Hoy & Nguyen 1994, as *C. quadristriatus*). Up to this time, there have been no recoveries and there was no evidence of establishment of this species (Huy & Nguyen 1994, 1997). Although *C. ingenuus* is predominantly an ectoparasi-

toid of citrus leafminer, Hoy & Nguyen (1997) reported that it was observed acting as a hyperparasitoid in Thailand (citing a personal communication from H. Browning). Subsequent to its initial release, laboratory tests were performed which confirmed that *C. ingenuus* could behave as a facultative parasitoid (Hoy & Nguyen 1997).

Recent field collections made in Homestead in southern Florida included specimens of *C. ingenuus.* The first record of this species was in November 1997, and they have been subsequently been found in January 1998. Collection data is listed at the end of the paper.

A total of 328 *C. ingenuus* were released in Florida (M. Hoy, pers. comm.), with the closest release sites to Homestead being Immokalee and Clewiston. Both of these sites are about 150 km north of Homestead, and each site received 40 *C. ingenuus* (M. Hoy, pers. comm.). These are the only documented releases of this parasitoid in Florida.

There are several possible explanations for the presence of *C. ingenuus* in southern Florida: the species could have been released in southern Florida without official knowledge; citrus infested with citrus leafminer and *C. ingenuus* could have been moved from central Florida to southern Florida; or this species became established in central Florida and has spread south. If its presence in southern Florida was the result of initial releases, it would appear that this species has now resided in Florida for over three years and is successfully established.

C. ingenuus has been released in many countries as a biological control agent of citrus leafminer, including Australia, Cyprus, Israel, Morocco, Oman, Syria, Tunisia, Turkey (Schauff et al., 1998).

The purpose of this note is to record of the presence of this species in southern Florida. Subsequent studies are planned to determine the extent of its impact.

Collection Data:

Florida, Dade Co., Homestead, November 1997, R.E. Duncan, ex. *Phyllocnistis citrella* on Citrus latifolia, 5 females, 17 males.

Florida, Dade Co., Homestead, January 15 1998, R.E. Duncan, ex. *Phyllocnistis citrella* on Citrus latifolia, 2 males.

Florida, Dade Co., Homestead, January 22 1998, R.E. Duncan, ex. *Phyllocnistis citrella* on Citrus latifolia, 3 females, 17 males.

Voucher specimens of these collections have been placed in the United States National Museum, Washington, Florida State Collection of Arthropods, Gainesville, and The Natural History Museum, London.

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BOOK REVIEWS

HALL, F. R., AND J. J. MENN (eds.). 1999. Biopesticides. Use and Delivery. Humana Press; Totowa, NJ. xiii + 626 p. ISBN 0-896-03515-8. Hardback. $119.50.

This book has 31 chapters contributed by 59 authors from Australia, Canada, China, France, Switzerland, UK and USA. Some are academicians, some are employed by industry, some by national and international research agencies, and some by national and international regulatory agencies. After an overview by the editors, the remaining chapters by other authors are grouped into 7 parts. Part 1, with 5 chapters, is called projections on opportunities for biopesticides in crop protection. Part 2, with 3 chapters, is on biofungicides. Part 3, with 10 chapters, is on bioinsecticides. Part 4, with 2 chapters, is on bioherbicides. Part 5, called "Other biorational technologies", has a single chapter on the use of pheromones. Part 6, with 4 chapters, is on registration of biopesticides, and addresses requirements in the USA and Europe. Part 7, with 6 chapters, is on management protocols.

As is obvious from Part 5, the book is not just about biopesticides. It reviews natural and synthetic plant-derived chemicals (such as azadirachtin and pyrethroids) and natural and synthetic chemicals derived from bacteria (such as delta entotoxins from *Bacillus*, and spinosyns from *Saccharopolyspora*); although these are chemicals, they sometimes are called "biorational pesticides." It discusses the use of synthetic pheromones and of transgenic crop plants; these both are sometimes called "other biorational methods." The discussion on transgenic plants is a small part of the book. The book thus reviews not only biopesticides (which are part of biological control), but also what elsewhere are termed "biorational pesticides" and "other biorational methods" (which are not part of biological control). A more descriptive title such as "Biopesticides and Biorational Methods" might have attracted wider sales from readers wanting a sourcebook of information on biorational methods. Transgenic crop plants are not part of biological control. But transgenic baculoviruses (when used as living organisms), which are discussed in this book, or transgenic entomopathogenic nematodes or predatory mites **are** part of biological control. The line between biological control and "biorational methods" is becoming more complicated but, wherever it is drawn, I find it useful that all this information should be revealed in one handy volume.

There has been voluminous coverage in the scientific literature of the development and management of transgenic plants, and voluminous criticism of this use in the scientific and popular press. The scientific literature is primarily concerned with trying to delay the development of resistance by pests to the toxins expressed in the transgenic plants, wherewith will be lost the natural regulation of pest populations afforded by naturally-occurring pathogens of pests. The popular press is more concerned with largely irrational fears by the public that consumption of genetically-modified food plants may somehow be harmful to human consumers. A third issue, in the realm of ethics, is the patenting of plant genomes by commercial interests. This book deals with all kinds of issues for the biopesticides and biorational methods, and is to be commended.

This book has something for many. For the research scientist who wants information, it deals with the origin, composition, and biological effects of biopesticides and biorational methods. For the agriculturist/horticulturist, it deals with availability, efficacy, and safety to the user of these materials. For the environmental specialist it deals with environmental safety. For commerce, it deals with opportunities in the production and marketing, as well as with governmental regulation in the USA, Europe, and elsewhere. And for everyone, it deals with human social issues and provides facts.

The editors and/or publishers have done their job well in curtailing verbosity, although some jargon has slipped by them (e.g., the word "impact", decried by the CBE Style Manual, rears its head). There are some typographical errors (e.g., "phereomone" in the Table of Contents and "*Beauveria bassisana*" p. on 47). Each chapter has its own References, and there is an 18-page Index. The Index manages to pick up the reference to *Beauveria bassiana* on p. 47, but erroneously attributes it to p. 147. It misspells the name *Paecilomyces fumosoroseus* which was given correctly on p. 32. It repeats the incorrect name "*Phthorminaea*" from p. 221. It omits the incorrect name "*Cnaphalocrosis medinals*" of p. 222 and the correct name *Xenorhabdus* of p. 273 and several other names. It does not consistently place names of genera and species into italics, and it introduces new errors such as "*Scapteriscus riobravis*" which should be *Steinernema riobravis*. The Index could have used more work, and the editors might have done better to include in it the name of the authority (describer) for each species mentioned, because the chapter authors have not done this consistently and have made errors. Better still, the editors might have provided the names of these authorities in a classificatory table of all organisms mentioned.

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TABER, S. W. 1998. The World of the Harvester Ants. Texas A&M University Press; College Station, TX. xvii + 213 p. ISBN 0-89096-815-2. Hardback. $34.95.

Steven Taber's book "The World of the Harvester Ants" is a breath of fresh air in a sea of otherwise dense technical literature. Taber obviously loves writing and studying harvester ants. The intersection of these interests is an engaging book written for amateur naturalists that is uncommonly easy to read, yet laced with details that even the most hardcore myrmecologist will find valuable. This book should be found in university, college, and public libraries throughout the western and southeastern United States wherever these conspicuous "red ants" occur.

Taber does a good job of summarizing the biology and ecology of *Pogonomyrmex* and *Ephebomyrmex* harvester ants using language that is accessible to virtually any lover of nature. As Taber notes, however, the downside of being brief and accessible is that this book does not provide a comprehensive review of the literature or a thorough description of what we know about the biology and life history of these ants.

The first chapter discusses Indian lore and other literature associated with harvester ants. The second chapter describes where harvester ants build nests and what they look like. Taber describes the trails and clearings around harvester ant nests and how some species seal off their nest entrances at night. In the third chapter, Taber discusses what harvester ants eat and how they gather their food. He also compares colony sizes of different harvester ant species and presents a history of Lincecum's hypothesis that red harvester ants intentionally plant and then harvest seed crops. The fourth chapter describes some of the interesting organisms that either eat harvester ants or live with them in their nests. The fifth chapter is an amalgam of topics ranging from pheromones and mating to chromosomes and oxygen consumption. The sixth chapter presents a cladogram of the morphological relationships among all *Ephebomyrmex* and *Pogonomyrmex* ants in North and South America. This cladogram shows a very clear division between *Pogonomyrmex* and *Ephebomyrmex*. Taber uses

this division to argue that ants in these two genera should not be lumped into a single genus. Unfortunately, Taber does not specifically address Bolton's lumping the two genera in his "Identification Guide to the Ant Genera of the World" (1994). The final chapter deals with the relationship between harvester ants and people and methods that can be used to control these ants.

Taber includes four appendices. The first is a list of all *Pogonomyrmex* and *Ephebomyrmex* species along with the meanings of their Latin names, something I found very interesting. The second appendix provides a key and tips for identifying all of the harvester ant species. The third appendix describes the characters used in the cladogram and the fourth appendix describes a new species, *Pogonomyrmex snellingi*.

A major strength of this book is that it provides excellent distribution maps for all of the species. This is especially useful because distribution maps for the *Ephebomyrmex* and South American *Pogonomyrmex* species have not been available until now. Furthermore, these maps are accompanied by good full-body line drawings of all 60 living species and one fossil species. Keys are also provided for all species, although users will find them challenging to use without a synoptic collection. This book also provides considerable information about the biology of South American harvester ants species that was previously only available in old, difficult-to-obtain, Spanish-language articles.

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