ASSESSMENT OF COTTON AS AN ALTERNATIVE HOST PLANT FOR THE BROWN CITRUS APHID, *TOXOPTERA CITRICIDA* (HOMOPTERA: APHIDIDAE)

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ABSTRACT

Seven populations of *Toxoptera citricida* (Kirkaldy) were sampled in central Florida sweet orange groves in 2001. All populations contained individuals that accepted cotton seedlings as a host in a no-choice situation; many of these matured and deposited nymphs that also developed and became reproductive on the same plant. Significant differences were noted among populations with respect to the proportion of nymphs accepting, maturing, and ultimately reproducing on cotton. Differences in aphid survival were largely a function of differences in host plant acceptance, rather than differential mortality on the plant. A significant proportion of the apterous adults maturing on cotton abandoned the plant without reproducing. Second and third instars transferred from laboratory colonies maintained on sweet orange were more accepting of cotton than were either first or fourth instars. Apterous adults accepted cotton at rates similar to second and third instars. Alate adults settled on cotton seedlings in greenhouse choice experiments and probed the plants, but none deposited nymphs. Alatae that matured on cotton readily accepted citrus for feeding and reproduction. It is concluded that cotton may be useful as a factitious host plant for rearing *T. citricida* in the laboratory, but field planted cotton is unlikely to serve as a reservoir of the aphid in regions where citrus is grown.

Key Words: *Gossypium hirsutum*, host plants, reproduction, survival, *Toxoptera citricida*.

RESUMEN

Siete poblaciones de *Toxoptera citricida* (Kirkaldy) fueron muestreadas en huertos de naranjas dulces en Florida central en 2001. Todas las poblaciones tenian individuos que aceptaron plantulas de algodón como una hospedera en una situación de una sola opción; muchas de estas maduraron y depositaron ninfas que tambien se desarrollaron y se reprodujeron en la misma planta. Diferencias significativas fueron notadas entre las poblaciones con respeto a la proporción de las ninfas que aceptaron, maduraron, y finalmente se reprodujeron en el algodón. Las diferencias en la sobrevivencia de los áfidos fueron mayormente en función de las diferencias en aceptar la planta como una hospedera, y no debido a la mortalidad diferential en la planta. Una proporción significante de los adultos ápteros maduraron en el algodón y abandonaron la planta sin reproducirse. Las ninfas en el segundo y tercero estadio transferidos de las colonias de laboratorio mantenidos en naranjas dulces fueron más receptivas al algodón que las ninfas en el primero o cuatro estadio. Los adultos ápteros aceptaron el algodón en las proporciones similares de las ninfas en el segundo y tercero estadio. Los adultos alados posaron sobre las plantulas de algodón en experimentos de selección en el invernadero y probaron las plantas, pero ninguno depositó ninfas. Los adultos alados que maduraron sobre el algodón aceptaron con rapidez el cítrico para alimentarse y reproducirse. Se concluye que el algodón puede ser útil como una planta hospedera facticiosa para criar *T. citricida* en el laboratorio, pero es poco posible que el algodón sembrado en el campo servirá como un refugio del áfido en regiones donde se siembra los cítricos.

The brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (BCA), is the primary vector of citrus tristeza virus (CTV), one of the important diseases of citrus world-wide (Meneghini 1946). Its importance as a pest of citrus derives from its high efficiency in transmitting this virus, rather than from any direct damage (Michaud 1998). The BCA has been present in Florida since 1995, but remains absent from other citrus-growing regions of the United States including Louisiana, Texas, Arizona and California. Although it has been present in Belize, Central America, since 1996 (Halbert 1996), the Yucatan Peninsula was not infested until 1999 (Michaud & Alvarez 2000). Northerly movement of the BCA has been also slow along the eastern seaboard of Mexico, and the major citrus-growing states of Tabasco and Veracruz remain uninfested to date. If and when further northerly movement

occurs, citrus plantings as far north as Texas could be heavily impacted as most citrus in the region, both north and south of the border, is planted on sour orange rootstock. Various strains of CTV cause "quick decline" of trees on sour orange and this rootstock must be abandoned wherever the virus and its vector are present together. An area-wide effort to identify and eliminate CTV-infected trees prior to the arrival of BCA is the best strategy for ameliorating the inevitable impact on the citrus industry.

The performance of BCA has been compared on various citrus varieties (Komazaki 1989) and related species of Rutaceae (Tang et al. 1999), but its ability to utilize non-rutaceous plants has not previously been explored. Although a substantial number of plants have been recorded as potential hosts for BCA (Michaud 1998), the actual role of these plants in supporting BCA populations in the field is unknown. It is suspected that many plants listed as hosts may represent mis-identifications of the aphid due to its similarity to the black citrus aphid, *Toxoptera aurantii* (Boyer de Fonscolombe), a related species with a very broad host range (Halbert & Brown 1996). Observations of BCA behavior suggest that anomalous host plant associations may arise when high-density populations 'overflow' from heavily infested citrus trees. Crowding in BCA colonies stimulates alate production (Michaud 2001), and large numbers of reproductive apterae also emigrate from crowded colonies (Michaud & Belliure 2000). These dispersing apterae ascend almost any other green plant adjacent to the source tree and often settle to feed. Residual nutrition acquired from the original host plant may then permit some limited reproduction to continue on the colonized plant, creating the semblance of host suitability. Thus, discrete field observations of host plant associations can be misleading and careful laboratory studies are required to determine whether a particular plant is truly a potential or suitable host.

The cotton plant, *Gossypium hirsutum* L., was first reported as an occasional host plant of BCA in southern Africa (Symes 1924) and later in Australia (Carver 1978). The present study was undertaken to evaluate the potential suitability of cotton as a host plant for BCA for two reasons. First, laboratory studies of the BCA and its biological control agents are hampered by the continuous requirement for citrus trees with new growth suitable for aphid colony growth and development. These are expensive to acquire and maintain, demand warm temperatures and intense supplementary lighting in order to produce new growth, and are susceptible to many other pests in a greenhouse environment. If the BCA could be reared effectively on a herbaceous host plant that could be planted from seed as required, laboratory studies of BCA biology and ecology would be greatly facilitated. Second, the close proximity of cotton plantings to citrus groves in many regions of Texas and California raises the question of whether or not cotton fields could potentially support reservoir BCA populations that could reinfest citrus, just as they now serve as a reservoir for *Aphis gossypii* Glover, another vector of CTV (Cisneros & Godfrey 2001).

The present study had three objectives: (1) to assess the general acceptability and suitability of cotton for various BCA populations in central Florida, (2) to test whether acceptance of cotton, and subsequent developmental performance, varies with the growth stage of the aphid colonizing the novel host, and (3) to determine whether alate aphids developing on citrus would colonize cotton and vise versa.

Materials and Methods

Variation among Populations in Acceptance of Cotton in No-Choice Experiments

Preliminary work conducted by Dr. A. Chow in Immokalee, FL indicated that BCA could be induced to feed on cotton provided that very young plants were provided and that relatively cool temperatures were maintained. Seeds of cotton, *Gossypium hirsutum* L., var "Suregrow", were planted individually in plastic cones (20 cm ht × 4 cm diam) filled with Metromix 500® potting soil. The cones were held at 24 ± 2°C in a climate-controlled greenhouse under natural light until germination. Following germination of the cotton, and before expansion of the first pair of true leaves, cones were individually labeled and a coating of Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI 40504) was placed around the inner rim of each.

Seven populations of BCA were sampled in sweet orange groves in seven distinct locations in Polk County, FL between 25-IX-2001 and 4-XI-2001 by collecting a single, heavily-infested citrus terminal from each grove and transporting it to the laboratory in a 500-ml ventilated plastic container. A series of 60 apterous, BCA 4th instars were selected from each sample under a low power stereo microscope and transferred with a sable hair brush in groups of 5 to each of 12 cotton seedlings. The seedlings were then placed in a growth chamber set to 16:8, L:D period, 75% RH, and a constant temperature of 20.0 ± 1°C. Each replicate was examined once every 24 h and the number of nymphs remaining on the seedling was recorded, as was the number dying in the Tanglefoot barrier. In addition, data were recorded on the number of nymphs maturing to the adult stage, the number of adults that reproduced, and the number of second generation nymphs that matured. The data were analyzed by one-way ANOVA (SPSS 1998) followed by an LSD test for separation of means ($\alpha = 0.05$).

Variation among Instars in Acceptance of Cotton in No-Choice Experiments

A stock colony of BCA was initiated from material field-collected in Polk County, FL, in March, 2002 and maintained on potted sweet orange, *Citrus sinensis* L., var. "Pineapple" at 24 ± 2°C in a climate-controlled greenhouse under natural light. Cotton seeds were planted individually in plastic cones, germinated in the greenhouse, and Tanglefoot was applied to the rim of the cone as above. Colonies of BCA were removed from the stock laboratory culture and the aphids separated according to stage (n1-n4 and apterous adults) under a 10× stereo microscope. For each growth stage, five aphids were transferred individually with a sable hair brush to a single cotton seedling in each of 20 replicates. Any aphid suspected to have sustained injury in the process of transfer was immediately replaced. Since adults are not reproductive for least 24 h following their last molt (Michaud 2001), pre-reproductive apterous adults were obtained by removing all adults from a stock BCA colony and, the next morning, harvesting all those that molted to adult overnight. All experimental replicates were maintained in a climate-controlled growth chamber under the same conditions as described above. Replicates were examined daily and the following information was recorded: the number of nymphs that settled and remained feeding on the plant after 24 h, the number maturing to the adult stage, and the number of adults that became reproductive. For transferred adults, only the number reproducing on the cotton was recorded. The data were analyzed by one-way ANOVA (SPSS, 1998) followed by an LSD test for separation of means ($\alpha = 0.05$). Survival of first instars was compared between experiments with a Chi-square, Goodness-of-fit test.

Alate Acceptance of Host Plants in Choice Experiments

Alate aphids were produced in high-density BCA colonies grown on potted sweet orange trees in the greenhouse (as above). Sweet orange seedlings and cotton seedlings were planted individually in plastic cones as above. Orange seedlings ca. 6 mo old with a single growing terminal were used in experiments; cotton seedlings were 2-4 days old. The experiments were performed in the greenhouse in wood frame cages (120 cm long by 65 cm wide by 80 cm high). Each cage was screened with white muslin on the side panels and had a clear plexiglass roof. In each trial (n = 12) the alate source consisted of a single 15-cm diameter pot containing a sweet orange plant with a mature BCA colony producing alatae. This alate source was placed in the center of a cage with 4 trap plants in plastic cones (2 cotton seedlings and 2 sweet orange seedlings) arranged equidistant (40 cm) in an alternating sequence around the source plant. After 24 h, the numbers of alatae settling and feeding on each of the trap plants were counted and the plants were replaced with cotton and citrus in reversed positions in the cage. In cases where alatae settled on a seedling, the seedling was isolated in another cage and examined on subsequent days to determine whether or not reproduction occurred.

Alate BCA were produced on cotton by transferring large numbers of reproductive apterous adults from the stock colony to the potted cotton seedlings and then moving them into a climate-controlled growth chamber under the same conditions as described above. The adult aphids were left to reproduce for a period of 48 h whereupon all adults were removed and first instar nymphs were left *in situ* to complete development. A total of 25 alatae produced on potted cotton seedlings were caged individually on flushed sweet orange terminals in the greenhouse in a muslin bag fastened with a twist-tie at the base of the twig. Observations were then made at 24 and 48 h to determine whether or not alates accepted the terminal and deposited nymphs.

Results

Variation among Populations in Acceptance of Cotton in No-Choice Experiments

All seven populations of BCA sampled contained some apterous fourth instars that accepted cotton as a host plant (Fig. 1), but there was significant variation among populations in the proportion of aphids that accepted the cotton seedling within the first 24 h ($F = 3.708$; 6,76 *df*; $P < 0.01$). There were also significant differences among populations in the number of individuals molting to adult ($F = 4.868$; 6,76 *df*; $P < 0.001$) and the number of adults reproducing ($F = 3.706$; 6,76 *df*; $P < 0.01$). Overall, a mean ± SEM of 21.4 ± 1.8% of aphids accepted cotton, 16.4 ± 1.6% molted to adult, and 10.2 ± 1.6% became reproductive. Population 2 had the highest proportion of individuals accepting, maturing and reproducing on cotton; only populations 1 and 6 had as many individuals accepting cotton, but their success in maturing and reproducing was significantly lower than population 2. A total of 69 aphids matured to the adult stage and 43 of these (63.2%) deposited at least one nymph before abandoning the plant. Apterous adults that became reproductive produced a mean (±SEM) of 13.9 ± 1.82 progeny (Fig. 2a) with no significant difference among populations in adult fecundity ($F = 0.904$; 6,26 *df*; NS) or in the number of progeny maturing ($F = 1.109$; 6,26 *df*; NS). The mean reproductive rate ranged from 0.4-2.2 nymphs/adult/day of reproduction (mean = 0.94 ± 0.23) and the overall maturation rate of 2nd generation nymphs was 33.8% (Fig. 2b).

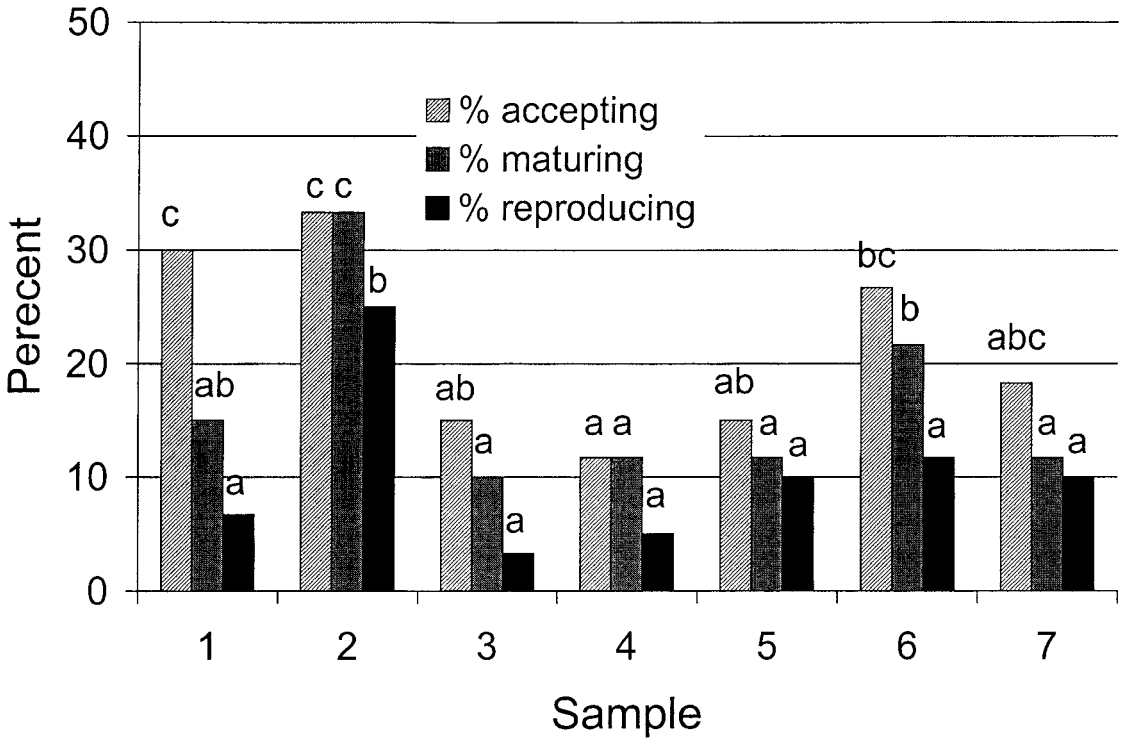

Fig. 1. Performance data for 4th instar *Toxoptera citricida* obtained from seven different populations in central Florida and transferred to cotton seedlings (N = 12), five per plant. "% accepting" = percentage of aphids feeding on the cotton seedling after 24 h, "% maturing" = percentage molting to adult, "% reproducing" = percentage depositing at least one nymph following molt to adulthood. Means in columns bearing the same letter are not significantly different among populations in a one-way ANOVA followed by LSD ($\alpha = 0.05$).

Variation among Instars in Acceptance of Cotton in No-Choice Experiments

There were significant differences among instars in the proportion maturing to adulthood when transferred from citrus to cotton seedlings ($F = 8.622$; 5,72 *df*; $P < 0.001$) and also significant differences in the proportion becoming reproductive ($F = 5.755$; 5,92 *df*; $P < 0.001$). Second and third instars were more likely to remain on a cotton seedling, survive to adulthood, and become reproductive than were either first or fourth instars (Fig. 3). Experiment-wide, a total of 266 of the aphids transferred as immatures molted to adulthood, and 190 of these (71.4%) deposited nymphs before abandoning the plant. Pre-reproductive adults transferred to cotton seedlings remained to reproduce with a frequency similar to second and third instars (Fig. 3).

Alate Acceptance of Host Plants in Choice Experiments

The proportion of nymphs maturing into alatae versus apterae on the source plants varied considerably under the conditions of these experiments, largely due to variation in both the number of apterous adults accepting the cotton, their distribution among the plants, and their reproduction during the 48-h period. High aphid density within colonies is the primary environmental factor influencing wing development in BCA (Michaud 2001), but high density colonies were difficult to achieve on cotton seedlings, leading to much lower rates of alate production than were achieved on citrus. A total of 186 alate aphids settled on plants and began feeding in the 12 replications of this experiment. Of these, 181 settled and fed on a sweet orange seedling and 5 settled and fed on a cotton seedling (Chi-square = 83.269, $P < 0.001$). Since observations were made only once every 24 h, it is possible that additional alates settled on cotton seedlings for shorter periods without remaining to feed. Whereas 98.3% of alates remained on orange seedlings long enough to initiate reproduction, all five that settled on cotton abandoned the plant within the following 24 h without depositing any nymphs. All 25 alatae that were reared on cotton and then caged individually on sweet orange terminals accepted the plant and began reproduction within 48-72 h.

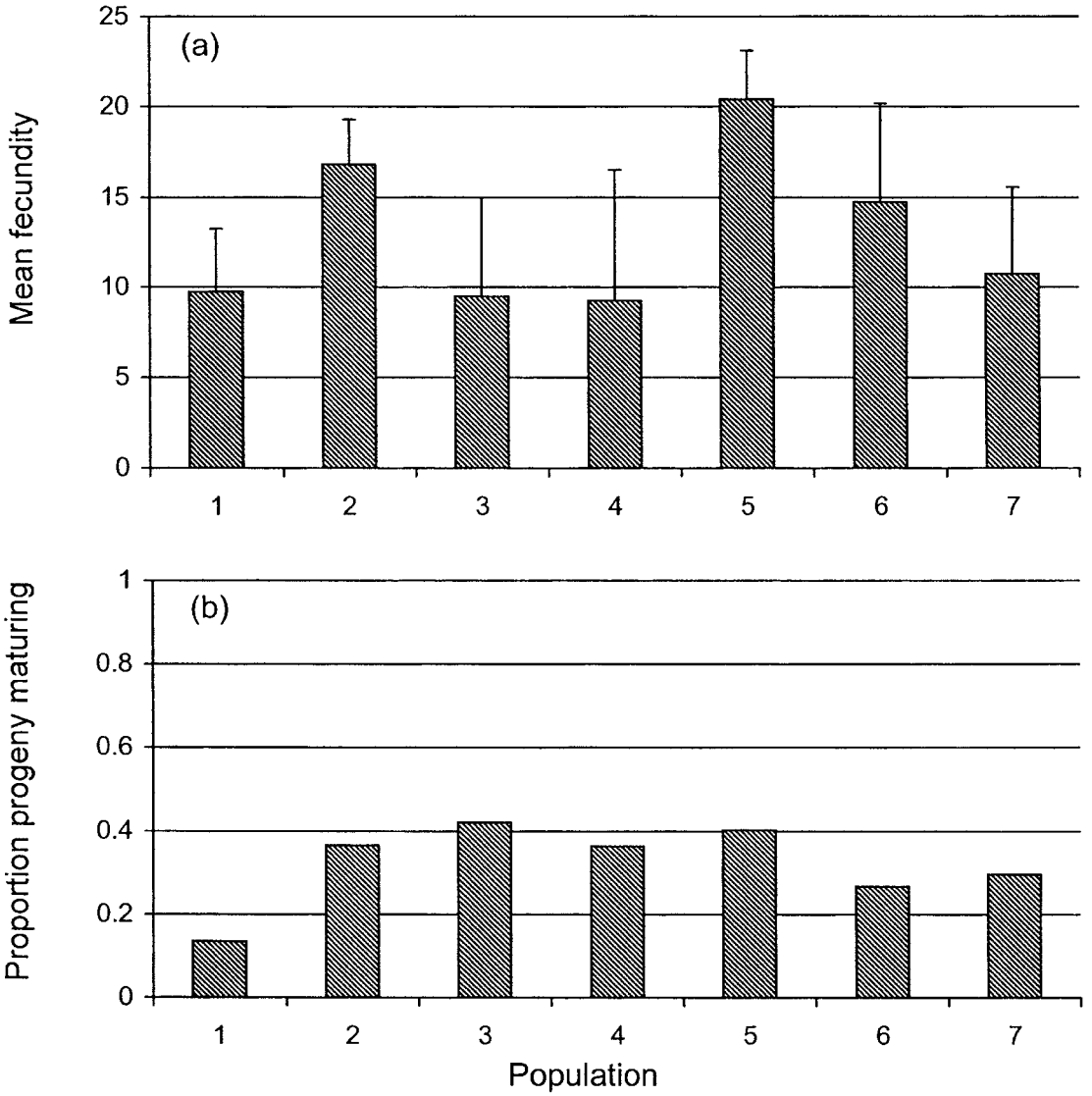

Fig. 2. Mean fecundities (+SEM) of *Toxoptera citricida* from seven field population that matured on cotton seedlings following transfer from citrus in the 4th instar (a), and proportions of the second generation nymphs that matured (b). There were no significant differences among populations (ANOVA, $P > 0.05$).

Discussion

The fact that all sampled BCA populations contained fourth instars able to feed, and ultimately reproduce, on cotton suggests that the physiological ability to utilize cotton as a host plant is probably a general characteristic of *T. citricida* populations. While it is not surprising that considerable variation exists among populations with respect to the acceptance of cotton, the potential significance of this variation remains obscure, given that reports of BCA attacking cotton in the field are evidently rare (Symes 1924; Carver 1978). However, BCA will also readily colonize *Murraya paniculata* (L.) Jack and *Malpighia punicifolia* L. under laboratory conditions (J. P. Michaud, unpublished) but rarely, if ever, utilizes these plants in nature.

If alatae are more selective of their host than are apterae, this could provide a partial explanation of why potential host plants such as cotton are rarely, if ever, utilized in the field. Alatae are physiologically very different from apterae in many ways. Their lower reproductive rate and longer lifespan (Takanashi 1989) may afford them more opportunity to be selective among host

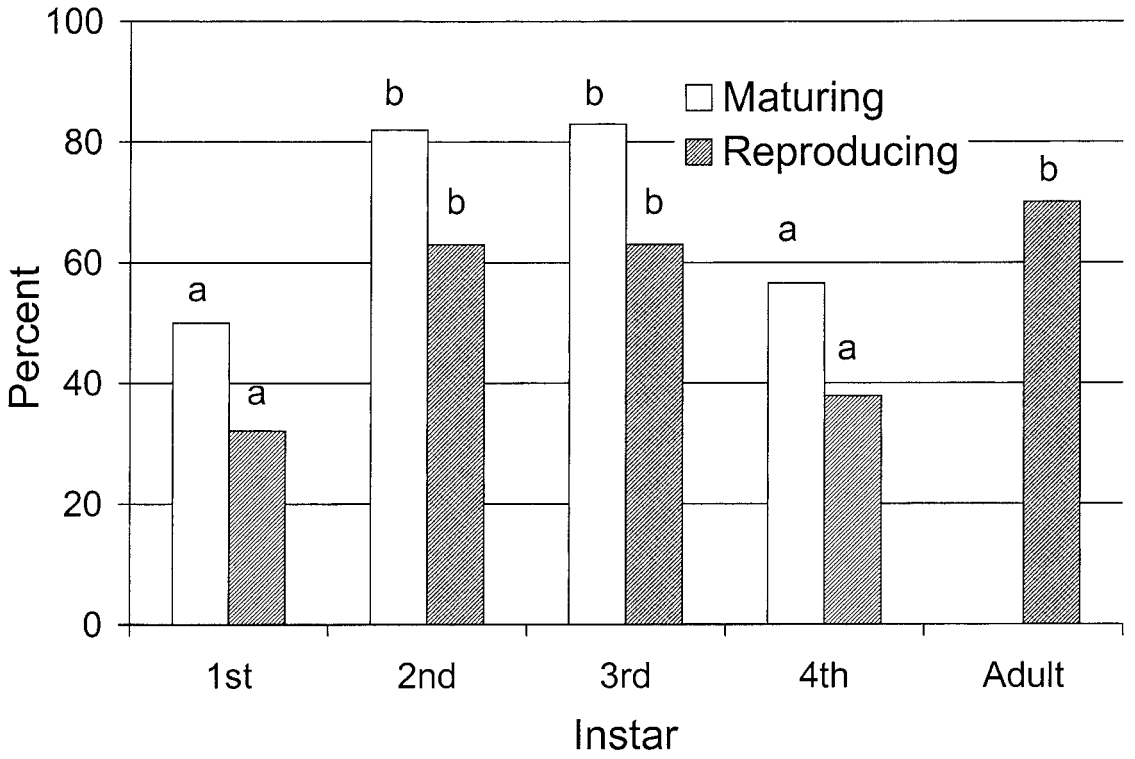

Fig. 3. Performance data for *Toxoptera citricida* transferred from sweet orange to cotton seedlings at various life stages. "% maturing" = percentage of aphids molting to adults, "% maturing" = percentage molting to adult, "% reproducing" = percentage depositing at least one nymph as an adult. Means in columns bearing the same letter are not significantly different among life stages in a one-way ANOVA followed by LSD ($\alpha = 0.05$).

plants. Apterae may often be constrained to accepting sub-optimal plants when dislodged from their primary host. Therefore, when reporting unusual host records for aphids it might be useful to distinguish between alate-founded versus apterous-founded colonies. Alate aphids are known to settle and probe on many non-host plants. For example, BCA alates probing soybean can contribute to transmission of soybean mosaic virus without ever colonizing the plant (Halbert et al. 1986). Similarly, many apterous-founded colonies on anomalous host plants may be chance events without ecological significance for the aphid population. An alate-founded colony (foundress with nymphs) is likely the best indicator of recurrent host plant utilization in nature.

It is important to note that aphid death in the first two experiments was almost invariably the result of aphids leaving the cotton seedling and dying in the Tanglefoot barrier, rather than simply expiring on the plant. Thus the differences observed in 'survival' and 'maturation' are largely a function of differential host plant acceptance, rather than differential mortality on the plant. Of all nymphs remaining on the cotton seedling for the first 24 h in the first experiment, more than three quarters matured and almost half became reproductive.

The variation in acceptance of cotton among different BCA instars was neither positively nor negatively correlated with aphid growth stage. If aphid nymphs increasingly 'acclimated' to cotton over the course of their development, one might expect early instars to perform better than later instars, but this was clearly not the case. Intermediate instars had higher acceptance and better performance on cotton than did either first or fourth instars. Better acceptance and survival of later instars was initially predicted on the assumption that more time spent feeding on the high quality host would yield better nutritional status and greater survival when transferred to a lower quality host. This would seem to adequately explain the results for early instars, but not for later instars. It is also possible that migration tendency is age- or size-dependent to some degree, since size and nutritional status could strongly influence survival during migration. Therefore, the pattern of acceptance observed in Fig. 3 is likely a function of various factors acting at different stages of development.

The foraging decisions of adult aphids necessarily concern the placement of their offspring, rather than being exclusively concerned with food consumption. A large proportion of the apterae maturing on cotton left the plant immediately upon molting to the adult stage (37.7% in the first experiment, and 28.6% in the second). Migration of reproductive apterae from BCA colonies has been documented in response to crowding (Michaud & Belliure 2000, 2001), but pre-reproductive apterae were not observed to emigrate under these conditions. In the present experiments, the emigration of many apterae immediately following the adult molt might reflect a decision to seek a more suitable host plant for progeny while adequate resources are still available. Although the majority of maturing apterae opted to allocate a fraction of their (potential) offspring to the cotton seedling before emigration, virtually all ultimately opted to abandon the plant. In the first experiment, only three reproductive apterae died on the seedling and remained hanging by their stylets; the remaining individuals were all recovered from the Tanglefoot barrier. Thus the estimate of fecundity is more reflective of the length of time apterous adults tolerated feeding on the cotton, rather than their intrinsic reproductive potential on the plant. Furthermore, the observed reproductive rate was only a fraction of that typically observed on citrus at a comparable temperature (Takanashi 1989) and is indicative of the relatively low suitability of cotton as a host for BCA.

Alate BCA frequently landed on cotton seedlings in the greenhouse but never remained on them long enough to deposit nymphs under the conditions of these experiments. While alatae placed directly on cotton seedlings and maintained at 20°C in a growth chamber will ultimately deposit some nymphs (J. P. Michaud, unpublished), this is not a meaningful observation since alatae seldom fly at this temperature, if they are able to fly at all. Thus colonization of cotton seedlings in the field by BCA alatae seems unlikely even under cool temperature conditions.

These experiments demonstrate that cotton seedlings may be colonized by apterous morphs of BCA, that BCA can develop and reproduce successfully on cotton under certain conditions, and that alatae developing on cotton will readily return to citrus. Cotton may, therefore, be useful as a factitious host plant for rearing BCA for purposes of scientific study, although colony growth rates are slower than on citrus. However, given that cotton is only acceptable during the seedling stage, and only to apterae under conditions of relatively low ambient temperature, it seems unlikely that there is much risk of cotton serving as a pest reservoir for BCA under field conditions.

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EFFECTS OF AN ENCAPSULATED FORMULATION OF LAMBDA-CYHALOTHRIN ON *NEZARA VIRIDULA* AND ITS PREDATOR *PODISUS MACULIVENTRIS* (HETEROPTERA: PENTATOMIDAE)

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ABSTRACT

Insecticidal effects of an encapsulated formulation of lambda-cyhalothrin on the southern green stinkbug *Nezara viridula* (L.) and one of its predators, *Podisus maculiventris* (Say), were investigated in the laboratory. Both pentatomids were exposed to the insecticide via contaminated drinking water and by residual contact. Nymphs and adults of *N. viridula* were more susceptible to the insecticide than nymphs of *P. maculiventris*, both by ingestion and contact exposure. For the respective ways of exposure, LC_{50} values calculated for *P. maculiventris* fourth instars were 30-190 times and 3-13 times higher than those of *N. viridula* fourth instars. Insecticidal activity of the pyrethroid by ingestion was 6-10 times greater against nymphs of *N. viridula* than against adults of the pest. In both the ingestion and residual contact experiments, nymphs of *P. maculiventris* recovered from initial knockdown. LC_{50} values for predator nymphs increased 1.7- to 2.7-fold between 24 and 48 h after the start of the experiment. Recovery from knockdown was not observed in *N. viridula*. The data from the current laboratory study suggest that encapsulated lambda-cyhalothrin may be effective for controlling the southern green stinkbug with little adverse effects on the predator *P. maculiventris*, but field experiments are needed to confirm this. Possible reasons for the differential toxicity of the insecticide to both pentatomids are discussed.

Key Words: lambda-cyhalothrin, pyrethroid, *Nezara viridula*, *Podisus maculiventris*, Pentatomidae, non-target effects

RESUMEN

Los efectos insecticidas de una mezcla encapsulada de lambda-cyhalothrin en la chinche hedionda verde de sur (southern green stink bug), *Nezara viridula* (L.) y uno de sus depredadores, *Podisus maculiventris* (Say), fueron investigados en el laboratorio. Ambos pentatómidos fueron expuestos al insecticida por medio de agua para beber contaminada y por el contacto del residuo del insecticida. Las ninfas y los adultos de *N. viridula* fueron más susceptibles al insecticida que las ninfas de *P. maculiventris*, por la ingestión y por la exposición por contacto. Para las respectivas formas de exposición, los valores LC_{50} calculados por las ninfas de *P. maculiventris* en el cuatro estadio fueron 30-190 veces y 3-13 veces más altos que los valores para las ninfas de *N. viridula* en el cuatro estadio. La actividad de la insecticida piretroide por ingestión fué 6-10 veces mayor contra las ninfas de *N. viridula* que contra los adultos de esta plaga. En ambos experimentos de ingestión y por contacto del residuo, las ninfas de *P. maculiventris* se recuperaron del derribo inicial. Los valores LC_{50} para las ninfas del depredador aumentaron 1.7 al 2.7 veces entre las 24 y 48 horas después de empezar el experimento. La recuperación del derribo inicial no fue observada en *N. viridula*. Los datos del estudio de laboratorio actual sugerieron que el lambda-cyhalothrin encapsulada puede ser efectivo para controlar la chinche hedionda verde de sur con pocos efectos adversos sobre el depredador *P. maculiventris*, pero se necesita llevar a cabo experimentos en el campo para confirmarlo. Se discuten las razones posibles para la toxicidad diferencial del insecticida para ambos pentatómidos.

The southern green stinkbug, *Nezara viridula* (L.), is a highly polyphagous pest that is widely distributed in the tropical and subtropical regions of the world (Todd 1989; Panizzi et al. 2000). This pentatomid causes important economic damage to various field crops, including soybean, beans, rice, corn, cotton and tobacco. In Europe, it has been found increasingly in greenhouses, where it attacks vegetable crops like tomato, sweet pepper, and eggplant. Control of this pest is based largely on the intensive use of chemical pesticides, including carbamates, organophosphates and some pyrethroids (Jackai et al. 1990; Ballanger & Jouffret 1997; Panizzi et al. 2000). For instance, in Brazil it was estimated that in the mid 1990s over 4 million liters of insecticides were used annually to control stinkbugs in soybean (Corrêa-Ferreira & Moscardi 1996). Such massive use of insecticides not only increases production cost, it may also affect populations of beneficial insects and trigger pest resurgence problems.

In several regions, efforts have been made to develop integrated pest management (IPM) pro-

grams against *N. viridula* (Panizzi et al. 2000). Biological control of the pest has focused mainly on the potential of parasitoids (Jones 1988). Releases of the scelionid egg parasitoid *Trissolcus basalis* (Wollaston) have successfully suppressed outbreaks of the southern green stinkbug in soybean (Corrêa-Ferreira & Moscardi 1996). Several arthropod predators also have an important impact on *N. viridula* populations. De Clercq et al. (2002) reported high predation rates by nymphs and adults of the predatory pentatomid *Podisus maculiventris* (Say) on the different life stages of the southern green stinkbug. This generalist predator is native to North America where it is commonly found in a variety of natural and agricultural ecosystems (De Clercq 2000). Although it appears to have a preference for larvae of lepidopterous and coleopterous insects, the predator frequently has been found in association with *N. viridula* in the southern United States (Drake 1920; Ragsdale et al. 1981; Stam et al. 1987). *Podisus maculiventris* has been used in European greenhouses since 1997 for augmentative biological control of caterpillar outbreaks, and predation on *N. viridula* may be an additional asset here for this beneficial insect (De Clercq et al. 2002).

In France, Ballanger & Jouffret (1997) reported effective control of *N. viridula* in soybean with foliar sprays of lambda-cyhalothrin. This contact and stomach insecticide belonging to the pyrethroid group has been used against a broad spectrum of pests in a variety of crops (Anonymous 2000). In 1999, a micro-encapsulated formulation (Zeon Technology™) of this pyrethroid was commercialised, offering reduced health hazards and an improved environmental profile (Ham 1999; Anonymous 2000). In the current study, insecticidal activity of an encapsulated formulation of lambda-cyhalothrin to *N. viridula* and its predator *P. maculiventris* was assessed in the laboratory. Both pentatomids were exposed to the insecticide via ingestion and residual contact. The implications of our findings for the control of the southern green stinkbug and the use of the predator *P. maculiventris* in IPM programs targeted against this and other agricultural pests are discussed.

Materials and Methods

Insect Cultures

A laboratory colony of *N. viridula* was established in 1999 with insects originating from field collections in France, Spain and Italy. Stinkbugs were fed on green bean pods (*Phaseolus vulgaris* L.) and sunflower seeds (*Helianthus annuus* L.). A culture of *P. maculiventris* was started in 1999 with specimens originating from a field collection in 1996 near Beltsville, Maryland, USA. The predators were fed mainly larvae of the greater wax moth, *Galleria mellonella* L. Colonies of all insects were maintained in growth chambers at 23 ± 1°C, 75 ± 5% RH and a 16:8 h light:dark photoperiod.

Chemicals

A commercial formulation of micro-encapsulated lambda-cyhalothrin (Karate Zeon®, 100 g/l) was obtained from Syngenta, Ruisbroek, Belgium.

Toxicity Bioassays

Exposure via ingestion. In these experiments, nymphs and adults of *N. viridula* and nymphs of *P. maculiventris* were exposed to insecticide through treated drinking water. Both pentatomids take up moisture in the absence of food and are regularly seen drinking even when food is available. Moisture can be supplied via plant materials, like green beans, but can also be provided as free water. Using gravimetric methods, we estimated that unfed newly molted fourth instars of *P. maculiventris* and *N. viridula* take up about 2 and 4.5 µl of free water, respectively, during a 24-h period.

Newly molted fourth instars and reproductively active adult female *N. viridula* were randomly collected from stock cultures and transferred to plastic petri dishes (9 cm diam) lined with absorbent paper. A replicate consisted of four insects in a dish for *N. viridula* nymphs, whereas adults were placed singly in dishes. Newly molted fourth instars of *P. maculiventris* were placed in petri dishes (9 cm diam) in groups of three. Each dish was supplied with a moisture source, consisting of a paper plug fitted into a plastic dish (2.5 cm diam). The paper plug was saturated with 2 ml of the insecticide in tap water. Control groups were supplied with tap water alone. At least 20 nymphs or 10 adults were tested with each of at least 10 concentrations. Choice of concentrations was based on preliminary range-finding tests. Test concentrations ranged from 0 to 200 mg a.i./l (11 concentrations) and from 0 to 100 mg a.i./l (10 concentrations) for *N. viridula* nymphs and adults, respectively, and from 0 to 800 mg a.i./l (13 concentrations) for *P. maculiventris* nymphs. Both pentatomids were exposed to the contaminated moisture source during a 1-wk period. To stimulate drinking behavior, the insects were not provided with food during the first 24 h. From the second day on, nymphs and adults of *N. viridula* were supplied with sunflower seeds as needed; predator nymphs were fed greater wax moth larvae *ad libitum*.

In range-finding tests, the ability of *P. maculiventris* in particular to recover from initial poisoning became apparent. Therefore, mortality counts were performed 1, 2, and 7 d after the initial treatment. Mortality percentages included dead and affected individuals. Insects were scored

as affected when they were incapable of coordinated movement upon prodding with a fine brush.

Residual exposure. To evaluate residual contact activity of lambda-cyhalothrin, fourth instars of both pentatomids were exposed by tarsal contact to dry residues on filter paper. Whatman No. 41 filter papers were fitted into petri dishes (9 cm diam) and the dishes were sprayed in a Cornelis spray chamber (Van Laecke & Degheele 1993). Each dish was sprayed with 2 ml of insecticide suspension in water, yielding a homogeneous spray deposit on the filter paper of approximately 5 mg/cm^2. For the controls, dishes were sprayed with 2 ml of water. The plates were left to dry for about 1 h before introducing the insects. A replicate consisted of a petri dish containing three *P. maculiventris* or four *N. viridula* nymphs. Twenty to 40 insects were tested per concentration, with a minimum of 10 concentrations. Concentrations were chosen on the basis of range-finding tests and ranged from 0 to 200 mg a.i./l (12 concentrations) for *N. viridula* nymphs and from 0 to 400 mg a.i./l (10 concentrations) for *P. maculiventris* nymphs. To stimulate food searching by the nymphs and maximize contact with the treated surface, no food or moisture were provided during the first 24 h. From the second day on, the insects were supplied with water and food. Water was supplied via a soaked paper plug in a 2-cm-diam cup. Nymphs of *P. maculiventris* were given freshly killed wax moth larvae and those of *N. viridula* were offered sunflower seeds. Mortality counts were made after 1, 2, and 7 d, allowing for the assessment of recovery after initial knockdown.

Data Analysis

Mortality of the test insects after 1, 2, and 7 d was corrected for control mortality by Abbott's formula (Abbott 1925). Lethal concentration values and their 95% confidence limits were calculated from probit-regressions with POLO PC (LeOra Software 1987). All concentrations tested were used for LC-calculations; numbers (n) given in the footnotes of Tables 1 and 2 thus reflect actual numbers of insects tested and used in probit-regressions.

Results

In our laboratory setup, there was no indication of a repellent or antifeedant effect for encapsulated lambda-cyhalothrin. In ingestion assays, both *P. maculiventris* and *N. viridula* were regularly observed to suck on moisture sources contaminated with varying concentrations of the compound. In the residual contact experiment, nymphs of both pentatomids were usually on the treated surfaces (filter paper, petri dish walls) and only occasionally on the untreated lid.

Nymphs and adults of *N. viridula* were more susceptible to the insecticide than nymphs of their predator *P. maculiventris*, both by ingestion (Table 1) and contact exposure (Table 2). For the respective routes of exposure, LC_{50} values calculated for *P. maculiventris* fourth instars were 30-190 times and 3-13 times higher than those of *N. viridula* fourth instars. Further, insecticidal activity of the pyrethroid by ingestion was 6-10 times greater against nymphs of *N. viridula* than against adults of the pest. Lethal concentration values calculated for *N. viridula* nymphs were lower for ingestion exposure than for contact exposure, suggesting that lambda-cyhalothrin was more active by ingestion than by tarsal contact with dry residues. However, some of the nymphs in the ingestion bioassays were partially or fully in tarsal contact with the moist plug when drinking, so these insects may have been exposed to the insecticide both via ingestion and via contact with wet residues. Differences in biological activity due to exposure routes were less apparent in the predatory pentatomid *P. maculiventris.*

In both the ingestion and residual contact experiments, nymphs of *P. maculiventris* recovered from initial knockdown. LC_{50} values for predator nymphs increased 1.7- to 2.7-fold between 24 and 48 h after the start of the experiment. Recovered predators were able to attack prey and feed normally. Comparisons of the LC_{50} values for *P. maculiventris* fourth instars after 2 and 7 d, however, show that some of the individuals that appeared to have recovered after 2 d died before reaching the fifth stadium when they were continuously exposed to contaminated drinking water or filter paper during a 7 d period. At 23°C, optimally fed fourth instars of both pentatomids usually reach the next stadium in 4-5 d (De Clercq & Degheele 1992). There was little or no recovery from knockdown in *N. viridula*. Here, LC_{50} values after 1 and 2 d were generally similar and further decreased with exposure time. Likewise, slopes were equal after 1 and 2 d ($\chi^2, P > 0.05$), but were significantly increased after 7 d of exposure. Only in the ingestion study with adults, LC_{90} values suggest there may have been some recovery at the upper end.

Discussion

In our experiment, both nymphs and adults of *N. viridula* were susceptible to encapsulated lambda-cyhalothrin. However, nymphs suffered about 5 times greater mortality when exposed by ingestion than by residual contact. For the control of *N. viridula*, the practical significance of ingestion exposure may be limited given that pyrethroids have no systemic properties. It is unlikely that the insect would ingest the compound in the field, except when it would drink from fresh spray deposits. Although lambda-cyhalothrin is widely recommended for the control of a broad range of insect pests, including stinkbugs, there are few studies reporting on the insecticidal effects of this pyrethroid on the southern green stinkbug. Ac-

TABLE 1. TOXICITY OF AN ENCAPSULATED FORMULATION OF LAMBDA-CYHALOTHRIN TO *N. VIRIDULA* AND *P. MACULIVENTRIS* BY INGESTION AFTER 1, 2, AND 7 DAYS.

Insect	LC value[a]			χ^2 (*df*)	Slope ± SE
	LC_{10}	LC_{50}	LC_{90}		
			1 day		
Nezara fourth instar	0.32 (0.12-0.61)	4.61 (3.12-6.50)	66.98 (39.35-147.46)	8.46 (8)	1.10 ± 0.11
Nezara female adult	2.79 (0.42-6.06)	28.70 (15.29-82.31)	295.72 (96.63-646.70)	8.03 (7)	1.26 ± 0.25
Podisus fourth instar	20.85 (12.67-29.68)	144.53 (116.50-181.80)	1001.83 (671.00-1776.99)	7.56 (10)	1.52 ± 0.15
			2 days		
Nezara fourth instar	0.30 (0.10-0.60)	3.83 (2.47-5.7)	49.43 (28.48-116.13)	10.72 (8)	1.15 ± 0.11
Nezara female adult	2.41 (0.13-6.19)*	40.04 (18.14-253.32)*	663.79 (141.99-1,798,890)*	13.84 (7)	1.05 ± 0.23
Podisus fourth instar	28.00 (16.29-40.8)	248.97 (193.15-340.43)	2214.20 (1279.44-5112.59)	7.77 (10)	1.35 ± 0.15
			7 days		
Nezara fourth instar	0.12 (0.05-0.22)	0.97 (0.64-1.32)	7.64 (5.57-11.63)	2.14 (8)	1.41 ± 0.16
Nezara female adult	1.36 (0.05-3.66)*	10.26 (3.92-26.16)*	77.23 (29.10-2164.97)*	24.40 (7)	1.46 ± 0.25
Podisus fourth instar	33.25 (20.83-46.08)	183.28 (146.88-234.81)	1010.36 (673.73-1838.99)	12.09 (10)	1.73 ± 0.17

[a]LC values and slopes in mg a.i./l; LC-values are followed by 95% fiducial limits except when marked with an asterisk (*) where 90% fiducial limits are given.
n = 456, 137, and 411 for *N. viridula* fourth instars, *N. viridula* females and *P. maculiventris* fourth instars, respectively.

TABLE 2. TOXICITY OF AN ENCAPSULATED FORMULATION OF LAMBDA-CYHALOTHRIN TO *N. VIRIDULA* AND *P. MACULIVENTRIS* BY RESIDUAL CONTACT AFTER 1, 2, AND 7 DAYS.

Insect	LC value[a]			χ^2 (*df*)	Slope ± SE
	LC_{10}	LC_{50}	LC_{90}		
			1 day		
Nezara fourth instar	4.96 (2.02-8.08)	19.92 (13.79-27.18)	80.02 (53.53-160.92)	22.23 (9)	2.12 ± 0.20
Podisus fourth instar	15.92 (3.39-28.64)	66.63 (42.88-93.82)	278.78 (169.62-981.19)	13.48 (7)	2.06 ± 0.30
			2 days		
Nezara fourth instar	4.75 (3.13-6.41)	19.06 (15.80-22.62)	76.50 (60.05-105.97)	8.21 (9)	2.12 ± 0.20
Podisus fourth instar	26.45 (1.27-51.00)	178.84 (106.72-1038.60)	1209.01 (392.16-482,011)	17.54 (7)	1.54 ± 0.29
			7 days		
Nezara fourth instar	4.80 (2.98-6.38)	11.29 (9.21-13.15)	26.55 (22.20-34.79)	3.38 (9)	3.45 ± 0.49
Podisus fourth instar	43.80 (10.62-66.79)*	147.81 (111.93-252.08)*	498.82 (278.24-4031.84)*	13.75 (7)	2.43 ± 0.53

[a]LC values and slopes in mg a.i./l; LC-values are followed by 95% fiducial limits except when marked with an asterisk (*) where 90% fiducial limits are given.
n = 416 and 255 for *N. viridula* fourth instars and *P. maculiventris* fourth instars, respectively.

cording to Ballanger & Jouffret (1997) and Gouge et al. (1999), the pest can be adequately controlled in soybean with non-encapsulated lambda-cyhalothrin at 20-30 g a.i./ha. In topical application experiments on third instars of *N. viridula*, Baptista et al. (1995) reported LD_{50} values of 0.82-0.25 µg/g for a technical grade formulation of lambda-cyhalothrin 3-24 h after treatment; the pyrethroid was 20-40 times more active against the pest than monocrotophos.

Susceptibility of the spined soldier bug, *P. maculiventris*, to classical and novel insecticides has been studied to some extent (see De Clercq 2000 for a review). Besides direct and residual contact, predatory pentatomids can be poisoned by drinking contaminated free water or plant sap (in case of systemic compounds) or by feeding on contaminated prey (De Clercq et al. 1995). In the current study, *P. maculiventris* was less vulnerable to lambda-cyhalothrin than *N. viridula*, both in the ingestion and contact exposure bioassays. Based on lethal concentration values, nymphs of the predator were somewhat more susceptible to the compound by residual contact than by ingestion. The LC_{50} value for fourth instars of *P. maculiventris* exposed to lambda-cyhalothrin via ingestion was similar to that found in an earlier study for nymphs treated similarly with deltamethrin (158 mg a.i./l, Mohaghegh et al. 2000). In a number of studies, *P. maculiventris* and related asopines have demonstrated a better tolerance to pyrethroids than their lepidopterous prey (Yu 1988; Zanuncio et al. 1993; Picanço et al. 1996). Yu (1988) hypothesized that thickness and lipid content of the cuticle may affect the penetration rate of the lipophilic pyrethroids and may thus be responsible for differences in toxicity to the heavily sclerotized pentatomid predators and their soft-bodied caterpillar prey. The finding of Baptista et al. (1995) that lambda-cyhalothrin was 5-9 times more toxic to the velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner) than to *N. viridula* supports this hypothesis. It may, however, not explain the differences found in our study because *P. maculiventris* and *N. viridula* are both pentatomids with a similarly sclerotized cuticle. Different drinking rates explain only in part the observed differences in toxicity of lambda-cyhalothrin to the studied pentatomids by ingestion. Preliminary gravimetric tests indicated that unfed fourth instars of *N. viridula* ingested twice as much free water during a 24-h period than did those of *P. maculiventris*, despite similar body weights (approximately 12.5 mg). Alternatively, higher detoxification or excretion rates may be responsible for the lower susceptibility of *P. maculiventris* to lambda-cyhalothrin as compared to *N. viridula*. Pyrethroids are known to be metabolised in insects mainly by esterases and microsomal oxidases (Shono et al. 1979). Yu (1987, 1988) found, however, that these enzyme activities were

generally lower in the spined soldier bug than in its caterpillar prey. Likewise, first tests have shown about 8-fold higher esterase activities in the phytophagous pentatomid *N. viridula* than in the predatory pentatomid *P. maculiventris* (unpublished data). Recently, it has been shown that glutathione S-tranferases also may play a significant role in detoxifying pyrethroids (e.g., Kostaropoulos et al. 2001). In this context, it is worth noting that Yu (1987) reported about 2-fold higher glutathione transferase activity toward CDNB in the spined soldier bug than in its lepidopterous prey. Further studies on the pharmacokinetics of lambda-cyhalothrin are warranted to explain the difference in toxicity of the compound to the studied pentatomids.

A number of field studies have demonstrated adverse effects of lambda-cyhalothrin on various beneficial arthropods, including predatory heteropterans, although in some cases negative effects on field populations of natural enemies were transient (Pilling & Kedwards 1996; Cole et al. 1997; van den Berg et al. 1998; Al-Deeb et al. 2001; Stewart et al. 2001). Encapsulation of broad-spectrum insecticides, including pyrethroids, is aimed at improving their selectivity and suitability for IPM programs (Scher et al. 1998; Ham 1999). However, several studies comparing encapsulated and non-encapsulated formulations of various insecticides have shown highly variable results with a range of beneficial arthropods (see Pogoda et al. 2001 for references). Pogoda et al. (2001) reported that a micro-encapsulated formulation of lambda-cyhalothrin was as toxic as an emulsifiable-concentrate formulation of the compound to the oriental fruit moth, *Grapholita molesta* (Busck). These workers also found that the encapsulated formulation of lambda-cyhalothrin was less toxic than the emulsifiable concentrate to a pyrethroid-resistant population of the phytoseiid mite *Typhlodromus pyri* Scheuten but more toxic to a pyrethroid-susceptible population of the predator.

The current laboratory trials indicate that encapsulated lambda-cyhalothrin has a good insecticidal activity against the southern green stinkbug and is relatively safe to the predatory stinkbug *P. maculiventris*. However, field studies are in place to test further the selectivity of this insecticide toward *P. maculiventris*. Further research also is needed to determine if the studied encapsulated formulation of lambda-cyhalothrin is more selective toward *P. maculiventris* and other natural enemies compared with other formulations of the compound.

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ARE REPRODUCTIVE TACTICS DETERMINED BY LOCAL ECOLOGY IN *ROMALEA MICROPTERA* (ORTHOPTERA: ACRIDIDAE)?

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ABSTRACT

We tested whether reproductive tactics of a univoltine insect can be predicted by local ecology, specifically mean length of the frost free period (FFP) as a measure of the potential active season. We measured reproductive tactics and longevity for populations of the lubber grasshopper *Romalea microptera* (Beauvois) from Miami, Florida (FL; 365 days FFP), Lydia, Louisiana (LA; 280 days FFP), and Athens, Georgia (GA; 224 days FFP). Differences in local climate led us to predict that GA grasshoppers will have shorter interclutch intervals, fewer clutches, and shorter lifespan than FL grasshoppers, with LA grasshoppers intermediate in these traits. When reared in a common laboratory environment, longevity, total reproductive period, and number of clutches produced were not clearly related to FFP. Longevity and reproductive period of LA grasshoppers were significantly less than those of FL grasshoppers, and number of clutches produced by LA grasshoppers was less than that for the FL or GA grasshoppers. First interclutch interval was significantly greater for LA than for GA grasshoppers. Our data suggest that phylogenetic relationships among populations may be a better predictor of reproductive tactics in this species.

Key Words: age at reproduction; climate; clutch size; grasshopper; life history; longevity; seasonality

RESUMEN

Probamos si las táctica reproductivas de un insecto del univoltine se pueden predecir por ecología local, específicamente longitud del período libremente mala de la helada (FFP) como medida de la estación activa potencial. Medimos táctica y la longevidad reproductivas para tres poblaciones del saltamontes, *Romalea microptera* (Beauvois), Miami, Florida (FL; 365 días FFP), Lydia, Louisiana (LA; 280 días FFP), y Athens, Georgia (GA; 224 días FFP). Estas diferencias en clima local conducen a la predicción que los saltamontes de GA tendrán período entre las hornadas del huevos más cortos, pocos hornadas del huevos, y esperanza de vida más corta que saltamontes del FL, con los saltamontes del LA intermedios en estos rasgos. Cuando estaba alzada en un ambiente común del laboratorio, la longevidad, el período reproductivo del total, y el número de los hornadas del huevos producidos no fueron relacionados claramente con FFP. La longevidad y el período reproductivo de los saltamontes del LA eran perceptiblemente menos que los de los saltamontes del FL, y el número de los hornadas producidos por los saltamontes de LA era menos que eso para los saltamontes del FL o de GA. El primer intervalo del hornadas era perceptiblemente mayor para el LA que para los saltamontes de GA. Nuestros datos sugieren que las relaciones phylogenetic entre poblaciones puedan ser un predictor mejor de estos aspectos de táctica reproductivas en esta especie.

Translation provided by the author.

Latitudinal variation in life histories can be related to adaptation to local climate (Rowe & Ludwig 1991; Temte 1993; Hemborg et al. 1998; Johansson & Rowe 1999; Berkenbusch & Rowden 2000; Hatle et al. 2002). For a univoltine organism, age at first reproduction and duration of interclutch intervals are likely to be positively related to the duration of the active season (Roff 1992; Forsman 2001), because of time-constraints in areas with shorter active seasons. There is often a tradeoff between early reproduction and longevity (e.g., De Souza Santos & Begon 1987; Rowe & Scudder 1990; Kaitala 1991; Stearns 1992; Leroi et al. 1994; Rowe et al. 1994; Miyatake 1997; Frankino & Juliano 1999). This trade-off yields a prediction of reduced longevity and late-life reproduction in populations from areas with short active seasons, where early reproduction is advantageous.

Hatle et al. (2002) examined latitudinal variation and trade-offs in reproductive tactics during the first oviposition cycle for three populations of the univoltine Eastern lubber grasshopper, *Romalea microptera* (Beauvois), testing for the joint

relationships of latitude to age at first reproduction, somatic storage (body mass immediately after oviposition relative to initial mass), and clutch mass. All three populations differed in their multivariate responses for the three reproductive tactics we studied. This difference across populations was due primarily to age at first reproduction, secondarily to somatic storage, and less so to clutch mass. Age at first reproduction was least in Georgia (GA) (34.5 ± 1.2 days; mean ± SE), and significantly greater for Louisiana (LA) (38.5 ± 1.4 days) and Florida (FL) (41.5 ± 1.4 days) grasshoppers. Estimated somatic storage was greatest in FL and LA, and least in GA grasshoppers. Clutch mass was greatest in LA and GA, and least in FL grasshoppers. Thus, allocation of resources among these reproductive tactics is different across populations, in ways that could be adaptive for each local climate.

In the present study, we investigate interpopulation differences in number of egg clutches, interclutch intervals, period of reproduction, and longevity using the same lubber grasshopper populations used by Hatle et al. (2002). Differences in climate and potential active season duration for these populations are indicated by the differences in mean duration of the frost free period (FFP) for these locations: Miami, Florida (FL, 365 days FFP); Lydia, Louisiana (LA, 280 days FFP); and Athens, Georgia (GA, 224 days FFP) (Koss et al. 1988). Because of the shorter period potentially suitable for reproduction, we predict GA grasshoppers will produce clutches faster, with shorter interclutch intervals than FL grasshoppers. Because of the putative tradeoff of longevity and early reproduction, we also predict that GA grasshoppers should have a shorter lifespan. Based on climate, the number of clutches and longevity for LA grasshoppers should be intermediate between those for GA and FL grasshoppers.

Materials and Methods

Grasshoppers were shipped as young nymphs from our three source populations to our laboratory at Illinois State University (Normal, IL, USA). Each population was reared on a 14L:10D photoperiod and a corresponding 32:24°C thermocycle. This photoperiod was chosen to approximate those observed at each of the sites in mid-active season for the adult grasshoppers. Photophases of 14 h occur at Athens at approximately 25 July, at Lydia at approximately 7 July, and at Miami at approximately 26 June (the longest photophase observed at Miami is 13.75 h) (US Naval Observatory 2003). A 14-h photophase was used by Hatle et al. (2002) in a previous comparison of reproductive tactics of these same populations. Photoperiod affects reproduction in *R. microptera*, with females from south Florida (Luker et al. 2002) and north Georgia (R. Homeny & S. Juliano, unpubl.) altering reproductive tactics in response to short photoperiods (11.5 and 12.0 h, respectively) associated with autumn. Thus, a 14 h photophase, typical of mid-summer at all sites, provides a reasonable point of comparison. All grasshoppers were offered Romaine lettuce and oatmeal *ad libitum* throughout the experiment. For a laboratory colony of lubbers from south Florida, the first oviposition cycle (~35 d) involves first somatic growth and then reproductive growth. During the first ~10 d the primary oocytes are not vitellogenic, despite a ~50% increase in somatic mass (Sundberg et al. 2001). Hence, the nymphal stages appear to be relatively unimportant for acquiring nutritional resources for egg production, and we are justified in conducting a common garden experiment beginning with newly molted adult females.

After adult eclosion, males and females were reared separately. Every other day, males and females were randomly paired for mating. Mated females were placed on 1.0 kg of sand with ~7% water (by mass) for oviposition. The calendar date of first oviposition, and all subsequent ovipositions, was recorded for each female. We maintained mated females until death or until 25 September 2002, when we terminated the experiment.

Data on interclutch interval, number of clutches, and period of reproduction were analyzed by one-way ANOVA with multiple comparisons (REGWQ method PROC GLM, SAS Inst., Inc. 1990a) among population means when the overall ANOVA test was significant. Assumptions of normality and homogeneity of variances were met. Proportions of experimental females in the three populations remaining alive at the end of the experiment were compared by Fisher's exact test (PROC FREQ, SAS Inst., Inc. 1990b). Longevity for the three populations was analyzed by nonparametric survival analysis (PROC LIFETEST, SAS Inst. Inc. 1990b, Allison 1995). Pairs of populations were compared for proportions alive and for survival time distributions with two-group Fisher's exact tests and two-group nonparametric survival analyses, respectively, with a sequential Bonferroni correction at experimentwise $\alpha = 0.05$ (Rice 1989).

Results

Clutch Production

Mean number of clutches produced differed significantly among populations ($F_{2,54} = 13.50, P = 0.0001$). LA grasshoppers produced significantly fewer clutches than did GA or FL grasshoppers, whereas GA and FL grasshoppers produced similar numbers of clutches (Fig. 1A).

The interclutch intervals between first and second, second and third, and third and fourth ovipositions were determined for each population

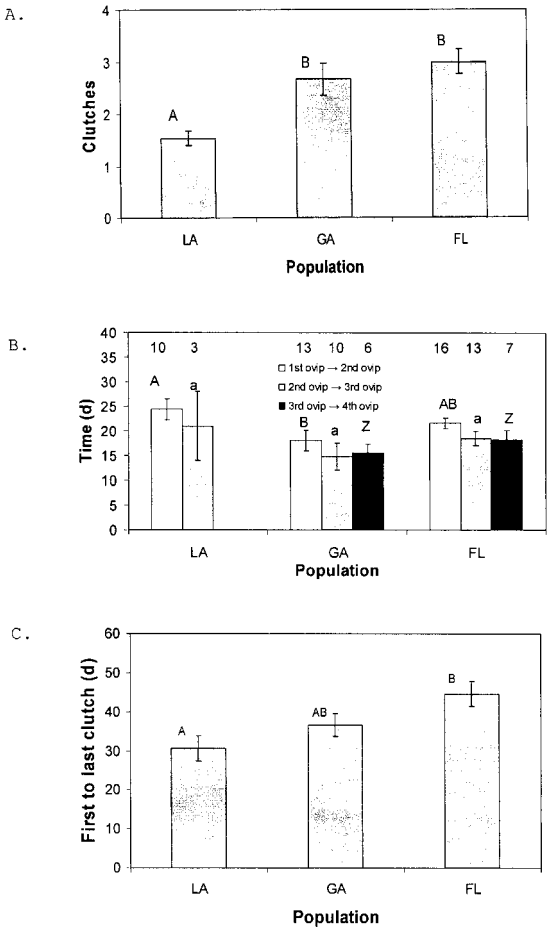

Fig. 1. Reproductive tactics of three populations of lubber grasshoppers, reared in a common environment. A. Number of clutches produced by each population. Means (± SE) for LA (N = 24), GA (N = 15), and FL (N = 18) grasshoppers associated with the same letters are not significantly different at $\alpha = 0.05$. B. Clutch intervals for three populations. Sample sizes are given at the top of the graph, above the corresponding mean. Within each interval, means (± SE) associated with the same letters are not significantly different at $\alpha = 0.05$. C. Time from each grasshopper's 1st clutch until its last clutch. Means (± SE) for LA (N = 10), GA (N = 13), and FL (N = 16) grasshoppers associated with different letters are significantly different at $\alpha = 0.05$.

(Fig. 1B). None of the interclutch intervals was significantly different at $P = 0.05$, but the interval from first to second oviposition for the LA vs. GA grasshoppers came close ($F_{2,36} = 3.12, P = 0.0564$).

Reproductive Period and Longevity

Reproductive period was quantified as the time from the first clutch until the last clutch (Fig. 1C). This period differed significantly among populations ($F_{2,36} = 4.86, P = 0.0136$) and was significantly less for LA grasshoppers than for FL grasshoppers. GA grasshoppers were intermediate and statistically indistinguishable from the other two populations.

The proportion alive at the end of the experiment differed significantly among the three populations ($P < 0.0001$). Pairwise tests indicated that the proportion alive for FL (0.63, N = 19) was significantly greater than that for LA (0.12, N = 25) and for GA (0.24, N = 17). Proportions alive for GA and LA did not differ significantly.

Survival distributions were quantified as the time from first clutch until death or the end of the experiment on 25 September. Individuals alive at the end of the experiment yielded censored observations, which are accounted for by PROC LIFETEST (see Allison 1995 for details). Survival analysis indicated significant differences in longevity among populations (Fig. 2). Pairwise tests indicated that longevity for FL was significantly greater than that for LA, and that GA was intermediate, and statistically indistinguishable from both FL and LA (Fig. 2). The majority of the FL individuals were still alive at the end of the experiment (Fig. 2). The early survivorship curves for FL and GA were very similar, indicating lower mortality than that for LA (Fig. 2), but later, mortality for GA accelerated and was more similar to that for LA (Fig. 2).

Discussion

Our prediction, based on local climate, that GA grasshoppers should have quicker clutch production and shorter lifespan than FL grasshoppers, and that LA grasshoppers would be intermediate, was not supported. Most reproductive tactics were roughly equal for FL and GA populations and longevity did not differ significantly. The most striking result in the data is the difference in reproductive tactics (number of clutches, reproductive period) and longevity between FL and LA grasshoppers.

We used the period from first clutch until death to estimate longevity. Hatle et al. (2002) found that these populations varied in the period from adult molt to first oviposition in a pattern partially consistent with variation of the frost-free interval (i.e., GA < LA = FL; see Introduction for means). In the present experiment, our measure of longevity did not include the period from adult molt to first clutch. Because of this, we have underestimated the difference in longevity between GA grasshoppers and LA and especially FL grasshoppers, and we have also underestimated the difference in longevity between LA grasshoppers and FL grasshoppers. Although the estimate of longevity we used is incomplete, the fact that it underestimates the difference between FL and LA grasshoppers strengthens our inference that these two populations differ in longevity.

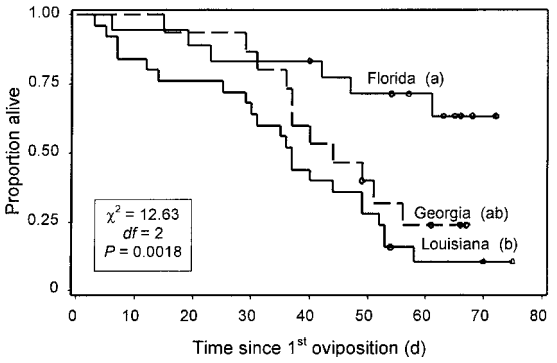

Fig. 2. Survivorship curves for three populations, beginning at first oviposition. Open points on the curves represent one or more censored observations. Overall χ^2 is for a nonparametric log-rank test of the null hypothesis of equivalent survivorship curves for FL (N = 18), GA (N = 15), and LA (N = 25). Curves associated with the same letters are not significantly different by pairwise log-rank tests (α = 0.05).

An estimate of total longevity for these three populations can be obtained by adding mean times to produce the first clutch reported by Hatle et al. (2002, see Introduction for values) and mean longevities from first oviposition recorded in the present study. Because of censoring, these mean longevities underestimate actual longevity, particularly for FL. Mean longevities from first oviposition for FL, GA, and LA are 51.5, 43.2, and 35.2 days, respectively, which yield estimates of longevities from eclosion of 93.0, 77.7, and 73.7 days, respectively. Thus, these estimates suggest it is FL that is unusual in its longevity and that despite a considerably greater apparent active season, LA grasshoppers have a lifespan as short as that for GA. These differences in longevity appear to be correlated with differences in first clutch mass (FL < GA = LA, Hatle et al. 2002), suggesting that longevity is indeed negatively related to early reproductive effort across populations.

Five years of field observations suggest that this population of LA grasshoppers senesces during the first week of September (J.D. Hatle, pers. obs.). Senescence occurs despite the fact that the mean temperature in Lydia, LA for 07 September is 27°C and mean rainfall for September is 144 mm (Weather.com 2003). Indeed, conditions seem to be ideal for lubbers during September in LA, and vegetation is still lush at this season. The mean temperature in Miami, FL for 07 September is 28°C and the mean rainfall is 160 mm. In Athens, GA the mean temperature on 07 September is 24°C and the mean rainfall is 98 mm. In contrast to their September senescence in LA, lubbers are present nearly the entire year in south FL, and all but the coldest months in north FL (Capinera et al. 2001), which has a climate very similar to south LA. In north GA, lubbers may be present into September (D. W. Whitman, pers. comm.), but are clearly declining in abundance during August (M. Brown, pers. comm.). It is unclear why this LA population of lubbers senesces in September, but based on our laboratory data, we propose that decreased survivorship of LA grasshoppers in September is a result of intrinsic factors that bring on senescence, rather than a result of increased disease or predation in this natural environment.

We find no evidence that interpopulation differences in interclutch intervals correlate with the duration of the FFP at these sites. The time required to produce the first clutch seems likely to be the most critical period with respect to laying multiple clutches before the end of the favorable season. The calendar dates of laying the second and third clutches are likely to be earlier if the first clutch is shorter. This may explain why we failed to find interpopulation differences interclutch intervals that correlate with the FFP, whereas Hatle et al. (2002) found interpopulation differences in the time required to produce the first clutch that did correlate with the FFP.

If local climate is not strongly related to these reproductive tactics, what does determine these fitness-related traits? Sequence analysis of mitochondrial DNA yielded a 69% probability that GA and FL populations are more closely related to each other than either is to the LA population (Mutun and Borst 2004). Thus, if reproductive tactics in this grasshopper are primarily associated with phylogenetic lineage, and not readily modified by local climate-driven natural selection (e.g., because genetic variation for these reproductive tactics is limited) we obtain alternative predictions: GA and FL grasshoppers will have similar reproductive tactics and longevity, and LA grasshoppers will differ from GA and FL grasshoppers. One of our results is consistent with this hypothesis. Grasshoppers from GA and FL produced a similar number of clutches, whereas LA grasshoppers produced a smaller number of clutches. Differences in interclutch intervals, time from first to last clutch, and longevity are not obviously consistent with either of these hypotheses.

Our results suggest that if these life history tactics are related to ecological conditions at each site, those conditions must involve more than active season duration, at least as it can be quantified by a crude measure like FFP. Alternatively, some differences in these life history tactics may in fact not reflect current adaptation, but rather, the phylogenetic constraints derived from the histories of different lineages. Because we have only examined three populations, our ability to correlate reproductive tactics with phylogeny is quite limited. Thus, at present we cannot distinguish between the hypotheses of more complex ecological determinants of reproductive tactics or phylogenetic constraints on reproductive tactics.

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FIELD OBSERVATIONS QUANTIFYING ATTRACTION OF THE PARASITIC WASP, *DIACHASMA ALLOEUM* (HYMENOPTERA: BRACONIDAE) TO BLUEBERRY FRUIT INFESTED BY THE BLUEBERRY MAGGOT FLY, *RHAGOLETIS MENDAX* (DIPTERA: TEPHRITIDAE)

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Abstract

The host foraging behavior of the larval parasitoid *Diachasma alloeum* (Muesebeck) (Hymenoptera: Braconidae) from natural populations was directly observed in a highbush blueberry, *Vaccinium corymbosum* L., plantation. More *D. alloeum* were observed alighting on blueberry fruit clusters infested with *Rhagoletis mendax* Curran larvae than were observed alighting on uninfested blueberry fruit clusters 80 cm away. Approximately equal numbers of *D. alloeum* alighted on uninfested blueberries that were mechanically damaged versus undamaged. The majority of *D. alloeum* females were attracted to host-infested blueberries 15 to 21 days after *R. mendax* females had oviposited into fruit. Female *D. alloeum* spent more time alighting on *R. mendax*-infested blueberry fruit clusters than on uninfested blueberry clusters 80 cm away. There was no difference in the duration of time spent by *D. alloeum* on mechanically damaged versus undamaged uninfested blueberries. The data herein are an initial step toward elucidating the cues mediating microhabitat selection by *D. alloeum* in blueberries.

Key Words: Conservation biological control, alighting behavior, *Diachasma alloeum*, *Rhagoletis mendax*

Resumen

El comportamiento del parasitoide larval *Diachasma alloeum* (Muesebeck) (Hymenoptera: Braconidae) por la busqueda del hospedero para alimentarse en poblaciones naturales fue observado directamente en plantaciones de mora azul, *Vaccinium corymbosum* L. Se observaron un mayor número de *D. alloeum* posando sobre los racimos de la fruta de la mora azul infestados con larvas de *Rhagoletis mendax* Curran de los que fueron observados posando sobre los racimos de fruta de mora azul no infestados separados por 80 cm de distancia. Aproximadamente números iguales de *D. alloeum* posaron sobre las moras azules no infestadas que fueron dañadas por la maquinaria agrícola versus las no dañadas. La mayoría de las hembras de *D. alloeum* fueron atraidas a las moras azules infestadas con el hospedero 15 a 21 días después que las hembras de *R. mendax* ovipositaron en la fruta. Las hembras de *D. alloeum* pasaron más tiempo posando sobre racimos de fruta de la mora azul infestados con *R. mendax* que en los racimos de la mora azul no infestadas separados por 80 cm de distancia. No habia una diferencia en la duración del tiempo que paso el *D. alloeum* sobre las moras azules dañadas por la maquinaria agricola versus las moras azules no dañadas y no infestadas. Los datos presentados aqui son un paso inicial hacia el aclaramento de las señales mediadoras para la selección del microhabitat hecho por el *D. alloeum* en la mora azul.

Hosts of insect parasitoids are often characterized by complex and patchy distributions making successful host location a major challenge for insect natural enemies (Hoffmeister & Gienapp 1999). Exploitation of chemical or visual cues associated with plants utilized by herbivorous hosts is known to increase host-searching efficiency of insect parasitoids (Vet & Dicke 1992; Godfray 1994; Vet et al. 1995). In addition, parasitoids are often attracted to damaged plants with cases of heightened attraction to plant damage created specifically by the herbivore host (Turlings et al. 1991; McAuslane et al. 1991, Henneman et al. 2002). The chemical cues released by herbivore-damaged plants and exploited by parasitoids include systemically released plant-volatile compounds (Dicke et al. 1993; Röse et al. 1996).

Several braconid species from the subfamily Opiinae are known to parasitize larval stages of Tephritidae (Wharton & March 1978). *Diachasma alloeum* (Muesebeck) occurs on hawthorn, *Crataegus mollis*, and apple, *Malus domestica* Borkhausen, in the northeastern U.S.A. and bordering regions of Canada and was thought to specifically attack the apple maggot fly, *Rhagoletis pomonella* (Walsh) (Glas & Vet 1983). Recently, *D.*

alloeum has also been reported attacking another member of the *Rhagoletis* sibling species complex, the blueberry maggot fly, *Rhagoletis mendax* Curran (Liburd & Finn 2003). Parasitization percentages of *R. mendax* larvae by *D. alloeum* collected from abandoned blueberry plantings in Michigan were extremely high, ranging from 30-50%. These rates of parasitization are higher than those known for *R. pomonella*, which range from 0.1 to 20.1% (Rivard 1967; Cameron & Morrison 1977; Maier 1982).

Only three detailed studies have been published on the behavior of *D. alloeum* attacking *R. pomonella* in hawthorns or apples. Boush & Baerwald (1967) reported on the courtship behavior and suggested the presence of a female-produced sex pheromone. Prokopy & Webster (1978) and later Glas & Vet (1983) analyzed the oviposition behavior of *D. alloeum* with specific interest in elucidating the stimuli involved in host-searching behavior. Visual orientation was found to play an important role for location of picked hawthorn fruit in laboratory assays and no difference in attractiveness was found between uninfested and *R. pomonella*-infested hawthorn fruit (Glas & Vet 1983). However, ovipositor probing activity and duration of stay were strongly influenced by the presence and movement of *R. pomonella* larvae feeding inside hawthorn fruit. The authors concluded that host movement within hawthorns was the prime stimulus for the location of host-infested fruit by *D. alloeum* (Glas & Vet 1983).

Recent studies with *Diachasmimorpha juglandis* (Muesebeck) have shown that females can distinguish between host-infested and uninfested walnut fruits before alighting (Henneman 1996, 1998). As they approach fruit, females hover close to the fruit surface for up to 1 sec before they alight or fly away, possibly assessing volatiles in order to decide whether to land (Henneman et al. 2002). Presence of fruit damage, however, rather than presence of larval infestation by *R. juglandis* larvae appears to produce the necessary cues for fruit choice by *D. juglandis* females (Henneman et al. 2002). Furthermore, both olfactory and visual cues are used by *D. juglandis* females to distinguish between infested and uninfested walnuts (Henneman et al. 2002).

To our knowledge, nothing has been published about the biology of *D. alloeum* in blueberry plantings. The current communication describes observations of the behavioral interactions of *D. alloeum* females with uninfested, mechanically damaged, and *R. mendax*-infested blueberries in an abandoned blueberry plantation. The specific objectives were to 1) determine whether blueberries infested with *R. mendax* larvae are more attractive to *D. alloeum* females than uninfested fruit, 2) determine whether mechanically damaged and uninfested blueberries are more attractive to *D. alloeum* females than undamaged and uninfested fruit, 3) document duration of visits and associated behaviors of *D. alloeum* on *R. mendax*-infested and uninfested blueberries in the field.

Materials and Methods

Research Site

Observational studies were conducted in the summer of 2001 in an abandoned plantation of highbush blueberry, *Vaccinium corymbosum* L. in Fennville, MI. The abandoned plantation was highly infested by *R. mendax* with approximately 45% of picked berries containing developing larvae in 1999 and 2000. In addition, this plantation was known to harbor a substantial population of *D. alloeum*. Parasitization rates of *R. mendax* collected from this plantation were above 50% in 1999 and 2000.

Insect Source

Rhagoletis mendax were reared from larvae collected from fruit of unsprayed blueberries (var. Jersey) from the plantation described above and from an organically managed plantation 3.2 km away. Flies were reared according to the protocol outlined in Liburd et al. (2003). Prior to testing, flies were maintained in aluminum screen-Plexiglas cages (30 × 30 × 30 cm) (BioQuip, Gardenia, CA) and supplied with water and food (enzymatic yeast hydrolysate and sucrose) (ICN Biomedicals, Inc., Costa Mesa, CA). Adults were kept at 24°C, 55-60% RH, under a 16:8 (L:D) photocycle.

Three weeks after removal of *R. mendax* puparia from 4°C (diapause), *D. alloeum* began emerging from more than 50 and 2% of puparia collected from the abandoned and organically managed sites, respectively. The parasitoids were identified by R. A. Wharton (Texas A&M University) and voucher specimens were deposited at Michigan State University (A. J. Cook Arthropod Research Collection).

Observational Study

Forty pairs of blueberry fruit clusters were selected for observation on 12 June before *R. mendax* emergence. Each pair of clusters was approximately 80 cm apart and each individual cluster contained 20-35 blueberries. All clusters were approximately 15-cm from the uppermost bush; this location within the blueberry bush canopy has been found to be the most effective position for trapping blueberry maggot (Liburd et al. 2000). At this stage of the season, blueberry fruit was still green and unripe. Experimental bushes were flagged and selected clusters were individually enveloped with 1 L translucent plastic bags that had been punctured with a pin multiple

times. Bags were positioned around blueberry fruit clusters such that berries did not directly contact the bag surface. The purpose of this bagging was to prevent native *R. mendax* from ovipositing into the selected berry clusters. On 19 June, we captured the first *R. mendax* on monitoring traps in the abandoned plantation. Twenty of the 40 bagged berry clusters were monitored from 25 June until 15 July to determine whether this bagging method interfered with normal berry development and to determine whether this technique successfully prevented *R. mendax* from ovipositing into berries. On each day, a single bagged cluster was randomly chosen for inspection. All fruit within that cluster were dissected and inspected for *R. mendax* larvae. In addition, on each day, two randomly selected clusters (15-20 berries) that had not been previously enveloped with a plastic bag were dissected for *R. mendax* larvae. No *R. mendax* larvae were found in berries that were enveloped by our plastic bags. In addition, berry size and color did not differ between bagged and unbagged berries. Among the unbagged clusters that were dissected, *R. mendax* infestation was first detected on 14 July.

The remaining 20 pairs of bagged clusters were used for the observational study. On 16 July, 10 of the 20 pairs of bagged blueberry clusters were randomly chosen for *R. mendax* infestation. Ten laboratory-reared and mated *R. mendax* females (10-15 days old) were introduced into one of the bagged blueberry clusters from each pair at 1200 hours. Introduced *R. mendax* were left in the bags for 24 h and then removed. Four of the 10 bags containing introduced *R. mendax* were observed for 1 h to confirm that flies were ovipositing into berries.

The other 10 pairs of bagged blueberry clusters were chosen for mechanical damage. The blueberries on one cluster of each bagged pair were mechanically damaged by making three equally spaced punctures in the skin of the berries with a 0-size insect pin. These manipulations resulted in 10 replicates of two paired treatments: 1) uninfested and undamaged berries versus *R. mendax*-infested berries, and 2) uninfested and undamaged berries versus uninfested and mechanically damaged berries. All other blueberries within a 1.5 m radius of each experimental pair were removed from bushes. The paired treatment clusters were 80 cm apart in a two-by-two design with the two treatments placed in alternate positions; each pair of treatment clusters was separated by at least 4 m.

Direct visual observations began 24 h after initial treatment manipulations were made and continued thereafter on every second day. Observations were conducted between 1230 and 1530 h. Two or more observers rotated among the ten replicates of each treatment pair conducting approximately 20-min observational bouts per location. Observations were terminated 31 days after the treatment manipulations were conducted.

During each period of observation, the plastic bags enveloping blueberry fruit clusters were removed and replaced immediately after observations were terminated. Also, native *R. mendax* were prevented from alighting on experimental clusters during observations. Observed events were spoken into a hand-held microcassette audio recorder by an investigator sitting or standing 0.75 m from the paired treatment clusters. Data recorded were: 1) landing by *D. alloeum* on berry clusters, 2) duration of visits on berry clusters, 3) oviposition into berries by *D. alloeum*. We attempted to collect observed *D. alloeum* with an aspirator after they oviposited and before they left experimental berry clusters. We estimate to have captured 70% of all visitors. The captured *D. alloeum* were taken to the laboratory and their identity was confirmed.

Statistical Analysis

Results of all dual-choice tests were analyzed by paired *t* tests (SAS Institute 2000). In all cases, significance level was $P < 0.05$. All ± values are SEM.

Results

Significantly more *D. alloeum* alighted per day on blueberry clusters that were infested with *R. mendax* larvae than on blueberry clusters that were uninfested (2.6 ± 0.5 and 0.3 ± 0.09, respectively). There was no significant difference between the number of *D. alloeum* alighting per day on blueberry clusters containing mechanically damaged and uninfested fruit compared with the number alighting on clusters containing undamaged and uninfested fruit (0.5 ± 0.8 and 0.5 ± 0.9, respectively). Blueberries that were infested with *R. mendax* larvae attracted the majority (64%) of *D. alloeum* females between 15 and 21 days after *R. mendax* females had oviposited into fruit (Fig. 1). There was no noticeable difference in attractiveness of mechanically damaged and undamaged fruit over time.

Female *D. alloeum* spent significantly more time alighting on *R. mendax*-infested blueberry clusters than on uninfested blueberry clusters (10.0 ± 1.1 min and 2.3 ± 1.2 min, respectively). There was no significant difference in the duration of time spent by *D. alloeum* on mechanically damaged and uninfested blueberries compared with undamaged and uninfested blueberries (1.1 ± 0.1 min and 0.9 ± 0.2 min, respectively). Of the 41 *D. alloeum* observed alighting on *R. mendax*-infested blueberry clusters, 34 were observed making a single ovipositional probe into blueberries. All of the *D. alloeum* that were observed ovipositing into berries performed "excreting"

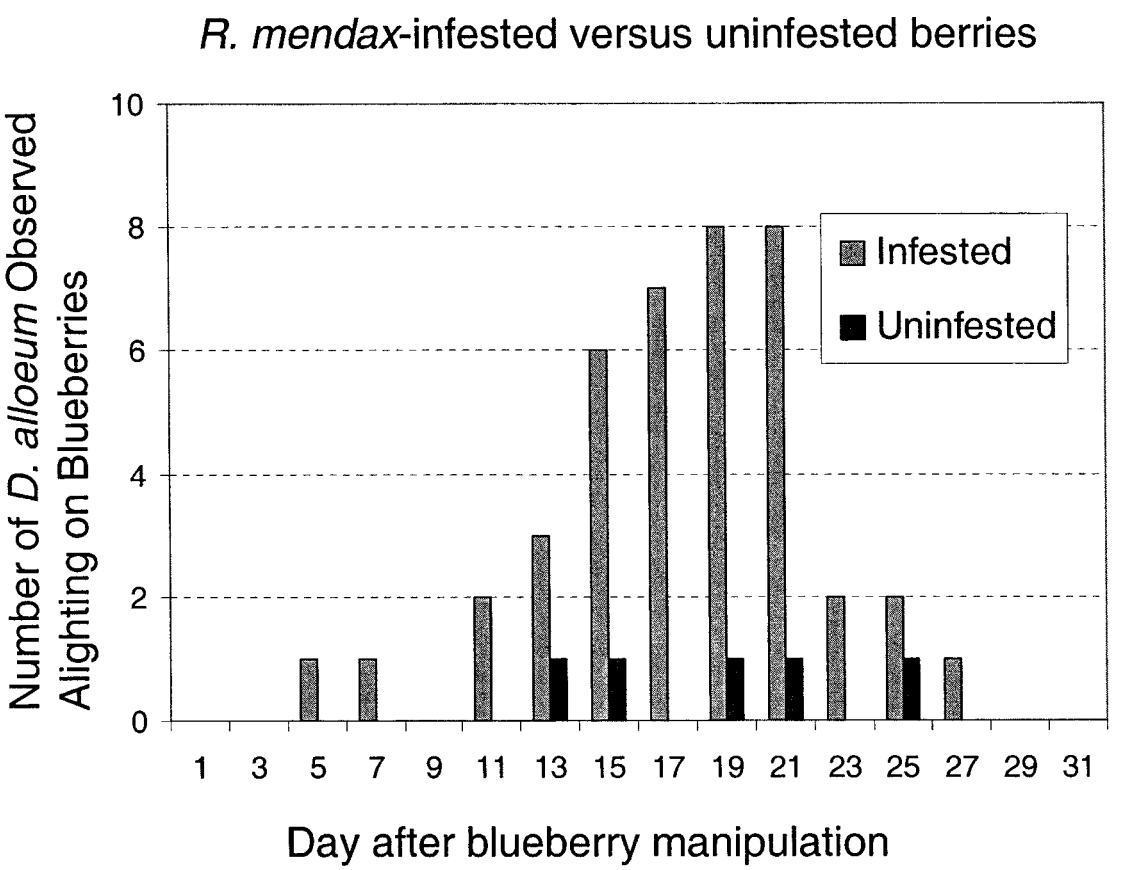

Fig. 1. Numbers of *D. alloeum* observed alighting on *R. mendax*-infested and uninfested blueberries spaced 80 cm apart every other day after *R. mendax* oviposition.

behavior directly thereafter as previously described by Glas & Vet (1983). Specifically, after ovipositing, these females walked on the blueberry dragging and dabbing their ovipositors on the fruit surface and excreting a clear fluid. None of the *D. alloeum* observed alighting on uninfested fruit attempted to oviposit.

Discussion

More host-infested blueberry fruit were visited by female *D. alloeum* than uninfested fruit suggesting that females have the capability of distinguishing *R. mendax*-infested berries prior to alighting. *D. juglandis* have also been shown to distinguish host-infested from uninfested fruits prior to alighting (Henneman 1996, 1998), relying on both visual and olfactory cues to make their decision (Henneman et al. 2002). *D. juglandis* distinguish host-infested fruit in the early stages of infestation (3-4 d after fly oviposition) as eggs are beginning to hatch (Henneman et al. 2002). In contrast to our results, previous laboratory studies comparing the attractiveness of *R. pomonella*-infested hawthorn fruit with uninfested fruit, showed that *D. alloeum* did not exhibit a preference and landed equally on both types of fruit (Glas & Vet 1983). However, the hawthorn fruit used in that study were field collected and infested by *R. pomonella* under laboratory conditions following a period of cold storage (Glas & Vet 1983). Thus, it is possible that the volatile profiles released by such picked and stored fruits may have differed from those of unpicked and *R. pomonella*-infested hawthorn fruit. It will be informative to determine whether *D. alloeum* distinguishes between *R. pomonella*-infested and uninfested hawthorn fruit under field conditions using unpicked fruit as was done in this study.

The behavior of *D. alloeum* documented in the current study varied in some respects from that previously reported in hawthorns. The majority of *D. alloeum* visits and ovipositions into *R. mendax*-infested blueberries occurred 15-21 days after female *R. mendax* had oviposited into the fruit. At this stage, the majority of *R. mendax* were likely in the second instar (Lathrop & Nickels 1932; Neunzig & Sorensen 1976). After the twenty-first day,

there was a dramatic reduction in the number of *D. alloeum* approaching and alighting on *R. mendax*-infested blueberries (Fig. 1). At this point, the majority of *R. mendax* larvae should have reached the third instar and were likely beginning to exit drying fruit to pupariate in the soil (Lathrop & Nickels 1932). In hawthorns, *D. alloeum* is known to attack the third (final) instar of *R. pomonella* (Glas & Vet 1983). In addition, *D. alloeum* spent less total time on blueberries during oviposition compared with hawthorns. On average, *D. alloeum* spent approximately 10 min on blueberries after alighting, while they spent anywhere from 18 to 140 min on hawthorn fruit during probing and oviposition bouts (Glas & Vet 1983). Furthermore, *D. alloeum* were observed making only one ovipositional probe per blueberry, while 1 to 5 ovipositional probes have been observed per individual *R. pomonella*-infested hawthorn fruit (Glas & Vet 1983). These differences in behavior of *D. alloeum* in blueberries versus hawthorns are possibly due to the differences in size and skin rigidity between blueberries and hawthorns. Given the smaller size and comparatively less rigid fruit skin of blueberries, it may be easier for *D. alloeum* to find and oviposit into a younger *R. mendax* larva in less time in blueberries than a comparably sized *R. pomonella* larva in hawthorns.

In the current study, more *D. alloeum* females landed on *R. mendax*-infested blueberries compared with uninfested berries, but not on mechanically damaged blueberries compared with the undamaged ones. This activity peaked 15-21 d after *R. mendax* had oviposited into blueberries. In contrast, *D. juglandis* females chose walnuts based on the presence of fruit damage rather than the presence of *R. juglandis* larvae inside the fruit (Henneman et al. 2002). However, in that study, mechanically damaged walnuts took on a distinctly different appearance (darkened) compared with undamaged walnuts, which was shown to influence fruit selection by *D. juglandis*. Color of host-infested walnuts is known to be an important visual cue mediating searching behavior of *D. juglandis* (Henneman 1998). In the current study, mechanically damaged blueberries did not appear different from undamaged berries. Furthermore, *R. mendax*-infested blueberries remained morphologically indistinguishable from uninfested berries for more than 20 days after *R. mendax* oviposition. It has been documented that certain female parasitic wasps exhibit an innate attraction to plant-released volatiles (Geervliet et al. 1996). Also, parasitic wasps are known to exhibit attraction to the host marking pheromone of tephritid fruit flies (Hoffmeister & Gienapp 1999). Based on the current results, we postulate that plant volatile compounds released by *R. mendax*-infested blueberries, but not mechanically-damaged fruit, provide an olfactory cue that attracts female *D. alloeum*. However, it is also possible that acoustic signals given off by chewing and tunneling *R. mendax* larvae within infested blueberries provide *D. alloeum* with an ovipositional stimulus.

Although labor intensive, our approach of conducting direct visual observations of *D. alloeum* responding to *R. mendax*-infested blueberries under authentic field conditions was indeed possible. Moreover, the data produced are an initial step toward elucidating the cues mediating microhabitat selection by *D. alloeum* in blueberries. The next step will be to determine whether *R. mendax*-infested blueberries release volatile profiles that differ quantitatively or qualitatively from those released by uninfested fruit. Finally, we hope to identify the relevant volatiles that may be involved in mediating attraction of *D. alloeum* to *R. mendax*-infested blueberries as has been done for other parasitoids (Turlings et al. 1991). Identification of plant volatiles attractive to *D. alloeum* may allow for recruitment of these beneficial insects in blueberry plantations, thereby improving biologically based management tactics for *R. mendax*.

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SEASONAL DEVELOPMENT OF *GRYLLOTALPA AFRICANA* (ORTHOPTERA: GRYLLOTALPIDAE) ON TURFGRASS IN SOUTH AFRICA

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Abstract

The population dynamics (in terms of seasonal development) of *Gryllotalpa africana* Palisot de Beauvois was documented for the first time in South Africa. An irritant drench (soapy water solution) was used to quantify life stage occurrence on turfgrass over a one-year period. Oviposition took place from early October (spring), with eggs incubating for approximately three weeks. Nymphs reached the adult stage from March (late summer) and most individuals overwintered in this stage. Adult numbers peaked in early September (early spring), declining through spring. *G. africana* was therefore univoltine in the study area. The adult population was female biased in spring. The smallest nymphs and adults (in relation to mean length) were collected in December (early summer), while the smallest nymphs (in relation to mean length) occurred in November (late spring).

Key Words: Univoltine, spring oviposition, life stage, turfgrass, mole cricket

Resumen

La dinámica de la población (en terminos del desarrollo estacional) de *Gryllotalpa africana* Palisot de Beauvois fué documentada por la primera vez en Africa del Sur. Una mojada irritante (una solución de aqua con jabón) fué utilizada para cuantificar la ocurrencia de los estadíos de vida en el césped durante un período de un año. La oviposición occurió desde el principio de octubre (la primavera), incubando los huevos por aproximadamente tres semanas. Las ninfas llegaron la étapa adulta desde marzo (al final del verano) y la mayoría de los individuos pasaron el invierno en este estadío. El número más alto de adultos se obtuvo en el principio de septembre (al principio de la primavera), y declinó atravéz de la primavera. Desde entonces, el *G. africana* fue univoltino en la área del estudio. Habian una inclinación viciada hacia las hembras en la primavera. Las ninfas y los adultos más pequeños (en relación al promedio de la longitud) fueron recolectados en diciembre (al principio de verano), mientras que las ninfas más pequeñas (en relación del promedio de la longitud) fueron recolectados en noviembre (al final de la primavera).

Gryllotalpa africana Palisot de Beauvois (African mole cricket) occurs only in Africa (Townsend 1983). The only account concerning the life cycle of this species is from Zimbabwe (Sithole 1986). Some notes on the species in South Africa were provided by Schoeman (1996) and Brandenburg et al. (2002).

Females lay 30-50 oval, white eggs in hardened chambers in the soil (Sithole 1986). Incubation period is temperature dependent, varying from 15-40 days. Nymphs feed on earthworms and roots of plants and under favorable conditions, develop through six instars with wing bud development visible in later instars (Sithole 1986). The nymphal period lasts three to four months. One generation per year is known (Sithole 1986). According to Schoeman (1996), there are approximately 10 instars of *G. africana* in South Africa and research by Brandenburg et al. (2002) showed that burrows of the African mole cricket are typically Y-shaped and range from 100 mm to 230 mm in length. The life and seasonal cycle of *G. africana* has not been investigated in South Africa and no reports on the seasonal development of *G. africana* on African turfgrass are available.

Life cycle, seasonal development and behavior documentations under the name *G. africana* include reports by the United States Department of Agriculture (1974) (U.S.A. potential introduction from Asia), Kim (1993, 1995) (Asia), Muralirangan (1979) (Asia), Tindale (1928) (Australia) and Goodyer (1985) (Australia). It is unknown if these studies refer to "true" *G. africana* from Africa.

Life cycle and seasonal development reports (including voltinism) for similar mole cricket species may vary significantly between geographical areas (Hudson 1987). In a specific area, different species and even different genera may show general life cycle similarity (including voltinism) (Frank et al. 1998). Therefore analysis of variations in life cycle, seasonality, and other factors for species occurring in climates similar to an area of interest may be more useful in estimating these parameters for a population than multiple studies of that species under a range of environmental conditions.

Materials and Methods

Infested kikuyu grass (*Penisetum clandestinum* Hochst ex Chiou) areas at Pretoria Country Club (25°47'16"S; 28°15'28"E) were flushed with soapy water (50 ml Sunlight® (Lever Ponds Pty Ltd., Durban) dishwashing soap/5 liters H_2O/m^2); this is a simple, inexpensive but effective surveillance technique (Short & Koehler 1979). Flushes started at noon with varying numbers of samples per sampling date (number necessary to collect ca. 100 crickets) with equal sampling intensity (10 liters soapy water) at each site over an annual period (Oct 2001-Nov 2002). Sampling was conducted every two weeks. Flushed areas were chosen at random within each site with the exception that no area was sampled twice over the duration of the experiment. Emerging crickets were captured, counted and total length measured from the posterior of the abdomen (excluding cerci) to the distal end of the labrum. Pronotal and abdominal lengths were also measured and recorded. Adults were sexed and females dissected to determine egg and oocyte presence for each sampling date. Oocytes were deemed mature (eggs) when covered by an egg shell (vitelline membrane and chorion). The long axis of mature eggs was generally longer than 2.5 mm. All sampled areas were under similar irrigation programs and soap flushing efficiency was assumed to be homogenous for adults and nymphs between and within sites throughout the study period. Immigration and emigration (especially through flight) were also assumed to be at equilibrium and not to affect absolute cricket sizes and life stage percentages during the study.

Deviation from an equal sex ratio was investigated by the two-tailed binomial distribution [Sokal & Rohlf 1997; "Statistica" Version: 5 (Statsoft, Inc. 1995)]. The Bonferroni method was used to lower the type one error probability for each comparison, resulting in an overall significance level not exceeding 0.05 in the entire series of tests (Sokal & Rohlf 1997).

Results

The life cycle of *G. africana* for each ontogenic stage as a percentage of the total population over an annual period is graphically presented in Fig. 1. Percentages were calculated by using adult and nymphal counts for a specific sampling date. Eggs were not sampled in the field therefore an 'estimate' of egg percentage on that date was calculated as equal to the mean first instar population percentage three weeks (mean egg hatch time) after that date. The egg percentage over time therefore only refers to fertilized eggs and may be subject to considerable variation, as incubation period is temperature dependant (Frank et al. 1998; Potter 1998). Life stage percentages were subsequently determined from the ontogenic ratio obtained. To obtain an annual presentation (from Nov 2001 to Oct 2002), data were therefore needed from Oct 2001 to Nov 2002. Fig. 1 shows 61% adults and 39% nymphs comprised the overwintering population (Jun-Aug). Patchy, relatively small samples (<40 individuals) were obtained during winter, which may contribute to the inconsistent results obtained during that period (Fig. 1). After overwintering, adult numbers (as a population percentage) peaked at 64% and diminished to 1% during Sep/Oct (spring) and Nov/Dec (spring/summer), respectively (Fig. 1). The egg population peaked at the end of Oct (spring) at 41.52%, further following the adult percentage inclination, but with some eggs laid until late Feb (Fig. 1). Oviposited eggs ranged from 2.5-3.5 mm in length. The graph of nymphal percentages showed an approximate direct inverse relationship with the adult-percentage-graph when no eggs were present (Fig. 1). High egg percentages were associated with the lowest nymphal percentages (Fig. 1). *G. africana* had a univoltine life cycle in the study area (Fig. 1). There is a lack of complete percentage overlap for each ontogenic stage at the beginning and end of the period (Fig. 1).

Mean monthly nymph and overall (adult and nymph) total length of *G. africana* for 12 months are shown in Fig. 2. First instars were 5.95 ± 0.218 mm (mean ± SD) long, with a midline pronotal length of 1.52 ± 0.054 mm (data not shown). The mean monthly nymphal length varied from 6.6 ± 2.56 mm to 25.8 ± 3.70 mm from Nov 2001 (first and second instars present) to Oct 2002 (late instars present), respectively (Fig. 2). Nymphs overwintered from early Jun 2002 when they were 23.0 ± 4.16 mm in length (data not shown), averaging 22.1 ± 3.9 mm over the month (Fig. 2). The mean monthly overall (adult and nymph) length was at a minimum (10.3 ± 6.51 mm) and maximum (31.1 ± 5.53 mm) in Dec 2001 and Oct 2002, respectively (Fig. 2). The mean monthly length of sampled nymphs and the total (nymphs and adults) population showed a relative decline during the winter (Fig. 2)). No females were sampled in Jan and Feb 2002, when one male in each month was flushed (resulting in no standard deviation values). Adult males and females were not distinctly segregated by mean length over monthly intervals, except for spring and early summer months, when females tended to be longer (data not shown). Males and females measured (mean ± SD) 35.91 ± 2.16 mm and 36.11 ± 2.40 mm, respectively, in Sep 2002, 31.75 ± 2.38 mm and 34.52 ± 3.94 mm, respectively, in Oct 2002, and in Dec 2001 30.83 ± 2.11 mm and 32.33 ± 1.84 mm, respectively. Males and females were at a maximum length of 36.7 ± 2.33 mm and 37.2 ± 1.85 mm, respectively in Nov 2001 and at a minimum of 30.8 ± 1.61 mm and 30.2 ± 1.27 mm, respectively in July 2002. The mean adult length over one year was 34.1 ± 3.87 mm, with a midline pronotal length of 7.8 ± 0.31 mm (data not shown).

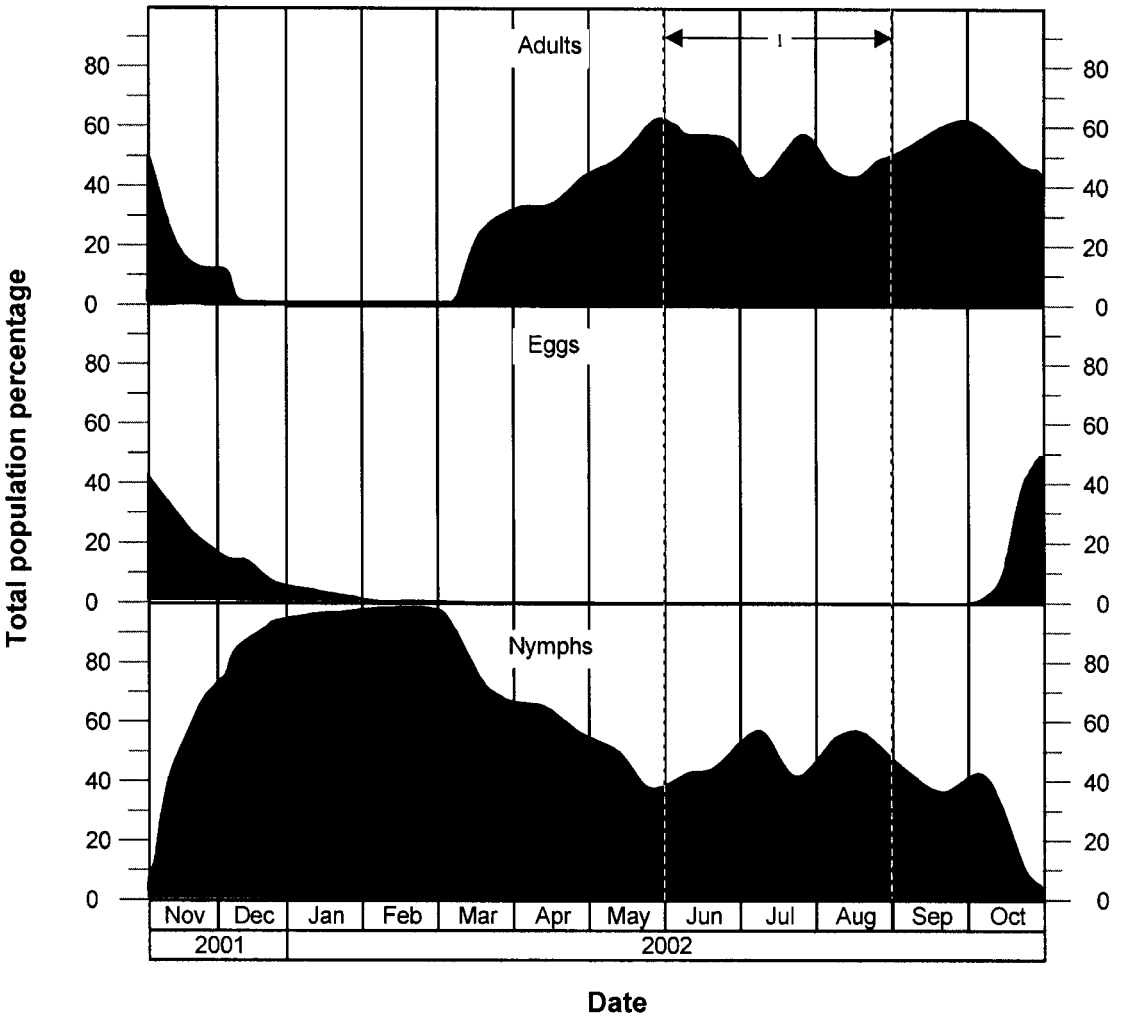

Fig. 1 The ontogenic stage population percentage of *Gryllotalpa africana* at Pretoria Country Club, Pretoria, South Africa from November 2001 to October 2002. Winter period.

Pronotal length was within the ranges reported by Townsend (1983). Development may be measured by other parameters than total length, but this study is also concerned with management, where a total length measurement is more practical and easily interpreted by turf managers. Management related sizes for other mole cricket species have also been reported in total length (Potter 1998; Brandenburg & Williams 1993).

Table 1 summarizes female reproductive activity and the sex ratio of *G. africana* per month over an annual period. Female oocytes started to develop in Apr and the percentage females with oocytes peaked in the winter months (Table 1). During Jul 2002, 92.3 ± 10.13% of females contained oocytes, a figure which was 20.0 ± 42.16% in Dec 2001. The mean percentage oocytes per female was highly variable in Dec 2001, but appeared to fit a declining pattern. Oocytes smaller than one mm in length were found in females from Apr 2002 to Aug 2002 they increased to 1.5-2.0 mm in Sept 2002 and 2.0-2.5 mm during Oct 2002, Nov 2001 and Dec 2001 (data not shown). Females containing eggs (2.5 mm to 3.5 mm long) were sampled regularly in Sep 2002, Oct 2002, Nov 2001 and Dec 2001, but peaked in Oct 2002 at 43.0 ± 0.00% of the female population. The highest number of internal eggs per female was found in Sep 2002 (38.4 ± 8.55), progressively declining to Dec 2001 (12.3 ± 9.78). The significance level for each sample was calculated as $P > 0.00217$ ($P > 0.05/23$ comparisons). Table 1 summarizes the mean (± SD) monthly percentage males of the adult population over 12 months. The adult field sex ratio was male biased one sampling date in May 2002 (date 1: 82.22% males, $P > 0.00002$, N = 45, date 2: 51.61% males, $P > 0.89908$, N = 62). Female bias (in the adult population) was found in

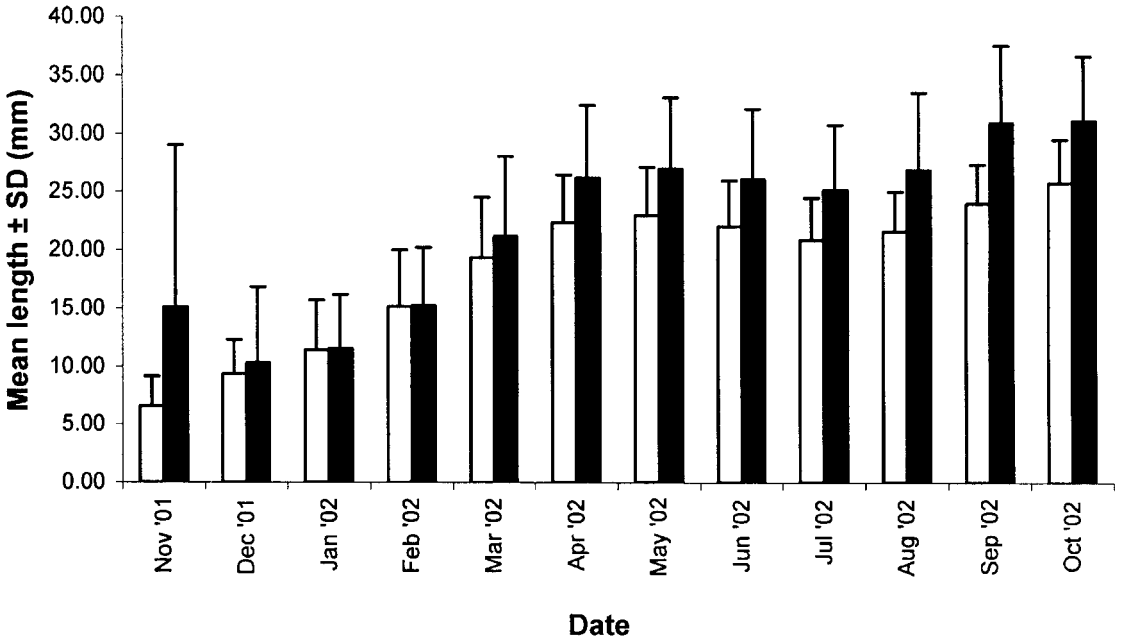

Fig. 2 The monthly mean total length (± SD) (from the posterior of the abdomen (excluding cerci) to the distal end of the labrum) of the nymph and total (adult + immature) population of *Gryllotalpa africana* at Pretoria Country Club, Pretoria, South Africa from November 2001 to October 2002. Total = black, nymphs = white.

both Aug 2002 samples (date 1: 12.12% males, $P > 0.00001$, N = 33, date 2: 24.53% males, $P > 0.00027$, N = 53). The first Sep 2002 adult sample was also female biased (date 1: 25% males, $P > 0.00023$, N = 56, date 2: 30.65% males, $P > 0.00316$, N = 62). The statistical results also indicated a female bias for the first Oct 2002 adult sample (date 1: 18.87% males, $P > 0.00001$, N = 53, date 2: 27.5% males, $P > 0.00643$, N = 40).

Field (Table 1) sex ratio data (as a male percentage, respectively) were normally distributed in the linear scale (Sokal & Rohlf 1997) for comparable months (Kolmogorov-Smirnov test, $P > 0.05$) ("Statistica" Version: 5 (Statsoft, Inc. 1995)).

Discussion

During the study period, vitellogenesis was observed from Sep and *G. africana* females laid fertilized eggs from Oct (mid spring). The highest number of fertilized eggs in the field was calculated as occurring during the end of Oct with some fertilized eggs laid until early Mar. Oviposition was in clutches (pers. obs.) but the subterranean nature of egg laying and clutches per female are unknown. The number of eggs per female and the adult population started declining from late Sep and reached a minimum in mid Dec (early summer). The monthly spring oviposition period was characterized by the longest females over an annual period that also comprised a significant proportion of the adult population. Female abdomen length appeared to increase with egg containment, as females were on average longer than males only at this time. However, female abdomen length did not appear to be linearly related to egg numbers. Absolute length may therefore not be the best measure to quantify adult size. Gender behavioral changes may also have influenced sampling results (lengths) over this period, but were assumed not to cause significant prejudice.

The data suggest mortality among males was high during late winter/early spring (causing a female bias). Migration through flight was not responsible for temporal gender bias in the field, as the monthly flight sex ratio was not significantly different to the monthly field sex ratio and also showed similar patterns. High male mortality after mating has been reported for other mole crickets (*Scapteriscus* spp.) with a univoltine life cycle (Brandenburg & Williams 1993; Buss et al. 2002), which suggests, if *G. africana* males show a similar tendency, that mating of *G. africana* occurred before spring in the present study. Mating may have occurred in autumn, which has been reported for univoltine *S. borellii* (Walker & Nation 1982), which also oviposit during spring (Frank et al. 1998). Further research (examining female spermathecae for sperm) will confirm mating period(s). The majority of adults were presumed dead (none recovered by soap flushing from the soil) by Dec (early summer), when the sex ratio approached 60:40. This suggests that high female mortality occurred after the oviposition period as reported for

TABLE 1. ADULT FEMALES CONTAINING IMMATURE AND MATURE OOCYTES (EGGS) (AS A FEMALE POPULATION PERCENTAGE, RESPECTIVELY), EGGS PER ADULT FEMALE AND THE ADULT SEX RATIO (AS THE PERCENTAGE MALES OF THE ADULT POPULATION) OF *G. africana* AT PRETORIA COUNTRY CLUB, PRETORIA, SOUTH AFRICA FROM NOV 2001 TO OCT 2002. (IMMATURE OOCYTES <2.5 MM AND MATURE OOCYTES (EGGS) >2.5 MM).

Date	Percentage females containing oocytes (mean ± SD)	Percentage females containing eggs (mean ± SD) (number of eggs per female) (mean ± SD)	Percentage males in population (mean ± SD)
November 2001	51.9 ± 16.94	35.71 ± 12.83 (23.4 ± 8.20)	36.1 ± 1.78
December 2001	20.0 ± 42.16	40.0 ± 21.09 (12.3 ± 9.78)	40.0 ± 16.24
January 2002	No females	No females	100 [a]
February 2002	No females	No females	100 [a]
March 2002	0.0	0.0 (0.0)	65.5 ± 11.92
April 2002	22.1 ± 14.67	0.0 (0.0)	53.0 ± 5.08
May 2002	36.6 ± 7.11	0.0 (0.0)	64.5 ± 15.18*
June 2002	91.8 ± 7.06	0.0 (0.0)	50.5 ± 0.63
July 2002	92.3 ± 10.13	0.0 (0.0)	36.6 ± 9.32
August 2002	80.0 ± 17.58	0.0 (0.0)	19.8 ± 6.07*
September 2002	62.7 ± 3.87	32.7 ± 8.60 (38.4 ± 8.55)	28.0 ± 2.83*
October 2002	45.6 ± 7.95	43.0 ± 0.00 (31.3 ± 9.15)	22.58 ± 0.04*

[a]Only one male and no females sampled (insufficient number for an inference).
*$P < 0.001$ in at least one sample (see results) (Two tailed binomial distribution, Bonferroni correction ($P = 0.05/23 = 0.002$)).

other mole crickets with a univoltine life cycle (Brandenburg & Williams 1993; Buss et al. 2002).

Eclosion (egg hatch) began in November, when distinctive first and second instars were abundant, and continued up to mid March. First instars were dorsally black with thin, white, horizontal, abdominal stripes, apterous and from personal observations, were the only active jumpers. Their total length was approximately 7 mm. Second instars were dorsally brown, apterous and up to 9 mm total length. All following instars were dorsally greyish-brown (adults and nymphs are light yellow on the ventral side) and resembled adults in appearance but were smaller and only developed wing buds in later instars. The relatively long oviposition period caused some instar overlap, as evident from standard deviation values for mean nymph absolute length. The overall (adult and nymph) mean absolute length was highly variable in November, but length was shorter with less variability in December, as a result of adult mortality over the two months. Nymphal development rate increased with relative warmer temperatures and the new generation adults appeared from late summer/early autumn. Adults have fully developed tegmina and hind wings and are capable of flight. The new generation adults consisted of more males during autumn, with a significant male inclination in May (although May samples were subject to relatively high variance). This suggests that males may eclose before females and then subsequently die earlier. The data indicate a minimum period of five months from oviposition to adult. The life cycle may, however, have been as long as eight or nine months if oviposition took place in late summer. The seasonal ontogenic stage occurrence was relatively similar in flush samples from across the Pretoria region (unpublished data).

Most nymphs completed their development by early June, when an over wintering phase was entered to the end of August, during which time individuals may have moved deeper down in the soil profile. During this period, small, patchy infestations (lowest density sampled during late July) were found in moist turf areas with relatively high soil temperatures (usually northern exposures). Sampling bias may have caused relatively high variability in life stage constitution during over wintering. Factors including behavioral changes, relative smaller samples prone to higher variability and/or destructive sampling may have contributed to the bias. Total length during winter samples showed a relative decline and also may have been due to sampling bias. Smaller (in relation to length) individuals sampled may have reflected a tendency of younger (and shorter) adults and nymphs to stay active as long as possible to attain a larger size (longer length) to increase their fitness during the following spring reproductive period. Larger males of *Scapteriscus* spp. produce louder calls and attract more females (Forrest 1980, 1983, 1991), while larger *Scapteriscus* spp. females produce three times more offspring and 1.5 times as many eggs per clutch than smaller females (Forrest 1986). The *G. africana* population became more adult biased during spring, when the development was completed. Adult length during spring was variable by month, but may support a contention by Forrest (1987), that as the spring reproductive period season progresses, a greater proportion of smaller individuals (of both genders) should mature because costs due to delaying reproduction increase.

There was annual variation (on a constant spatial scale) in the development of *G. africana*. Mean egg hatch in 2002 was 2 weeks later than in 2001. Soap flushes should, therefore, be con-

ducted weekly to quantify spatial and temporal variance (this is especially important to guide management practices).

The seasonal development of *G. africana* reported in this study is very similar to that reported for univoltine *Scapteriscus* spp. in the southeastern U.S.A. (Brandenburg & Williams 1993).

Preliminary studies indicate peak oviposition occurred a few weeks later on golf courses in the cooler, southern regions (Johannesburg), a pattern followed by some New World species (Brandenburg 1997; Potter 1998). Temperature therefore appeared to be an important factor influencing egg laying period in *G. africana*. Brandenburg (1997), however, found that timing and intensity of egg-laying and egg hatch do not seem to be closely related to soil temperature or the number of *S. vicinus* and *S. borellii* females captured in acoustic traps. Hertl et al. (2001) found a significant positive linear relationship between ovipositing females (number of eggs laid per female were constant) and soil moisture in *S. borellii*. Soil moisture may also influence oviposition in *G. africana*.

Preliminary studies also show that the proportion of adults in the population prior to overwintering might be smaller in the southern areas (Johannesburg). Adult overwintering proportions are variable (on a constant spatial scale) for *S. vicinus* (Brandenburg 1997), suggesting that values reported in this study may also be variable between years.

Some specific behaviors of *G. africana* were observed during the course of this study. Adults were found to be cannibalistic, especially at high densities. *G. africana* adults usually expelled a characteristic non-sticky, pungent smelling, dark brown fluid when handled, possibly as a deterrence or defense mechanism (pers. obs.). Other genera (*Neocurtilla* and *Scapteriscus*) and *Gryllotalpa* species (*G. oya*) also are known for secreting fluids that may be smelly and vary from a low to high viscosity (Baumgartner 1910; Tindale 1928; Walker & Masaki 1989).

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A NEW SPECIES OF *ODONTOMACHUS* ANT (HYMENOPTERA: FORMICIDAE) FROM INLAND RIDGES OF FLORIDA, WITH A KEY TO *ODONTOMACHUS* OF THE UNITED STATES

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ABSTRACT

The ponerine ant *Odontomachus relictus* n. sp. is described from specimens collected in scrub and sandhill habitats on several ancient sand ridges in Florida. It appears to be a relict species from dry periods in the Pleistocene. Workers are similar to the western species *O. clarus* Roger, but males of the two species are strongly divergent. Keys and natural history notes are provided for workers and males of the four *Odontomachus* species known from the U.S. Examination of males might help clarify the taxonomic status of *Odontomachus* of Central and South America.

Key Words: Florida endemics, arenicolous arthropods, *Odontomachus*, ants, Formicidae, Ponerinae

RESUMEN

La hormiga ponerine *Odontomachus relictus* n. sp., es descrita de especímenes recolectados en los hábitat de matorrales y de los bancos de arena en varias lomas de arena antíguas en la Florida. Parece ser una especie reliquia de los periodos secos del Pleistoceno. Los obreras son similares a la especie occidental *O. clarus* Roger, pero los machos de los dos especies son fuertemente divergentes. Se provee las claves y notas de la historia natural sobre las obreras y los machos de las cuatro especies de *Odontomachus* species conocidas en los Estados Unidos. La examinación de los machos puede ayudar a aclarificar el estatus taxonómico de los *Odontomachus* de centro y de suramerica.

Members of the genus *Odontomachus* are often common and conspicuous insects. They are relatively large for ants (length often around 8 mm), with elongate mandibles whose powerful snapping action is produced by massive muscles accommodated in bulging lobes on the head capsule (Fig. 1A, B). *Odontomachus* has achieved some fame for the speed of its mandibular snap, which occurs in 0.33-1.00 millisecond, the fastest animal movement known (Gronenberg et al. 1993). In spite of these formidable jaws, backed up by a sting strong enough to elicit a definitive reaction in humans, these ants are not particularly fierce, and are usually seen stalking slowly about singly on the surface of leaf litter. As might be expected, examples of such large and obviously interesting ants began to accumulate in collections at an early date, resulting in the naming of numerous species and forms. This proliferation of names was based to a large extent on what seemed to be a good array of useful characters, including pilosity, color, surface sculpture, and the shape of the mandibles and petiole.

Unfortunately, it appears that many of the characters used in diagnoses of *Odontomachus* species show intraspecific variation, resulting in large numbers of synonyms. Bolton's 1995 catalog of Formicidae includes 161 specific and subspecific names for extant *Odontomachus* species, 60 of which Bolton lists as valid names. Most of the credit for the simplification of nomenclature should go to Brown's 1976 review of the genus. Lineages that include variable species also may include cryptic species, and this seems to be true of *Odontomachus*. In the U.S., *Odontomachus* nomenclature was at its most austere following Brown's 1976 salutary pruning of the genus, resulting in one recognized species in the Southeast, *O. brunneus* (Patton), and one recognized species in the Southwest, *O. clarus* Roger. Deyrup et al. (1985) showed that there were three species in the Southeast: *O. brunneus*, *O. ruginodis* M. R. Smith (probably a relatively recent introduction to the area), and what appeared to be an isolated population of the southwestern *O. clarus*, restricted to arid dunes in central peninsular Florida. New evidence reveals that the southeastern species thought to be *O. clarus* is a different, undescribed species.

The purpose of this paper is to name this new species, to present an identification guide to the four U.S. species, to summarize the known natural history of all four species, to briefly cover the nomenclature of the species, and to indicate a few residual problems.

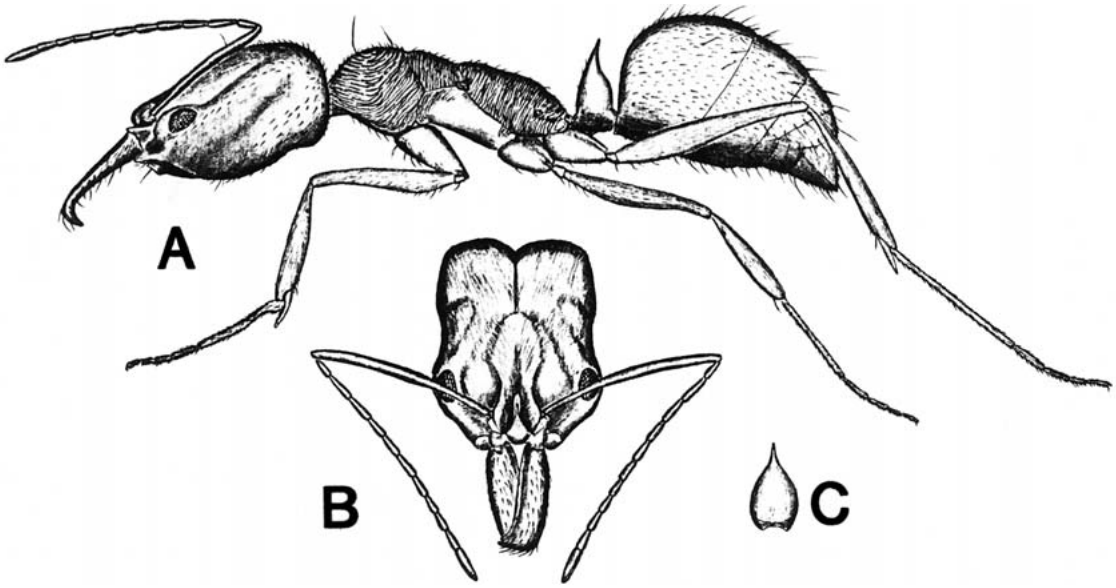

Fig. 1. *Odontomachus relictus*, new species, worker. A. Lateral view. B. Frontal view of head. C: Posterior view of petiole.

Classification and Diagnosis of *Odontomachus*

Family Formicidae, subfamily Ponerinae, tribe Odontomachini (Hölldobler & Wilson 1990). *Odontomachus* is a senior synonym of *Pedetes* Dalla Torre, *Champsomyrmex* Emery, and *Myrtoteras* Matsumura (Bolton 1995); these junior synonyms have not been used for more than 25 years, and there is no current confusion about these names.

Diagnosis (modified from Hölldobler and Wilson 1990)

Mandibles slender, elongate, attached near middle of anterior margin of head, abruptly bent inward at apex, widened apices expanded with three teeth arranged in a vertical series; third abdominal segment not differentiated by a constriction from the rest of the abdomen. Nuchal carina (the ridge delimiting the occiput) V-shaped, narrowed toward its median into a mid-dorsal groove; apophyseal lines present as convergent lines from the vertex of the head up to the nuchal carina.

The shape of the nuchal carina and the presence of apophyseal lines distinguish *Odontomachus* from the somewhat similar genus *Anochetus*. A simple character for separating *Odontomachus* from *Anochetus* in the U.S. is the shape of the petiole, ending in a dorsal spine or simple cone in *Odontomachus*, and in a pair of spines or a pair of angles in *Anochetus mayri* Emery, the only representative of its genus known from the U.S. (Deyrup 2002). *Anochetus mayri*, which is native to the West Indies and exotic in Florida, is smaller (4 to 5 mm in length) than the *Odontomachus* species considered here. *Anochetus kempfi* Brown (West Indies) is within the size range of local *Odontomachus*, but very slender, and with a two-spined petiole.

Odontomachus relictus Deyrup and Cover, **new species**

Diagnosis of worker (Fig. 1).

Distinguished from other U.S. *Odontomachus* by the following combination of character states: conspicuous striae present on basalar lobe (oval sclerite at dorsal posterior corner of mesopleuron); posterior side of petiole without transverse striae; appressed hair on first gastral tergite sparse, short, spaces between hairs often as wide as the length of hairs.

Description of holotype worker

Features visible in lateral view described from left side. Measurements in mm. Total length (length of head excluding mandibles + length of mesosoma + length of petiole + length of gaster): 7.48; head length: 2.12; width of head at rear margins of eyes: 1.80; width of head at widest part of occipital lobes: 1.62; length of left mandible: 1.20; maximum width of eye: 0.30; maximum width of clypeal area: 0.30; length of mesosoma: 2.67; length of petiole: 0.52; length of gaster: 2.17. Head: fine striae diverging from frontal lobes, covering frontal aspect of head, disappearing before occipital area, covering only the upper quarter of the extraocular furrow; posterior lateral corners, occipital area, underside of head smooth and shining. Mesosoma:

pronotum with roughly circular concentric striae, without longitudinal striae reaching the hind margin; mesonotum and propodeum with transverse striae; striae present on basalar lobe; mesopleuron smooth, shining, with longitudinal striae along dorsal and ventral margins. Petiole: apically spinose; in profile anterior face convex, posterior face bisinuate; posterior face in posterior view smooth and shining, without hairs. Gaster: shining, no surface sculpture except for minute punctures from which hairs emerge; first tergite sparsely covered with short, appressed, pale hairs, spaces between hairs usually as wide as length of hairs; first tergite with sparse, scattered, suberect long hairs, including an uneven subapical row. Color: head, antennae, mesosoma, petiole dark reddish brown, contrasting with blackish-brown gaster; legs dark yellow, contrasting with mesosoma.

Diagnosis of queen

Queens of the four U.S. species were examined. Diagnosis as in worker.

Description of a paratype queen

Measurements in mm. Total length (length of head excluding mandibles + length of mesosoma + length of petiole + length of gaster): 8.37; head length: 2.15; width of head at rear margins of eyes: 1.77; width of head at widest part of occipital lobes: 1.70; length of left mandible: 1.20; maximum width of eye: 0.27; maximum width of clypeal area 0.25; length of mesosoma: 2.75; length of petiole: 0.52; length of gaster: 2.95. Structural character states and color as in worker, except for occurrence of ocelli and expansion of the mesosomal dorsum (pronotum, mesonotum, scutellum) associated with flight; pronotum transversely striate, mesonotum longitudinally striate.

Diagnosis of male

Distinguished from other U.S. *Odontomachus* by the following combination of character states: ocelli very large, wider than the distance between the lateral ocelli and the eyes (Fig. 2A); body color medium brown, antennae yellowish.

Description of a paratype male

Measurements in mm. Total length (length of head excluding mandibles + length of mesosoma + length of petiole + length of gaster): 6.66; head length: 1.07; width of head at widest part, including eyes:1.45; length of mesosoma: 2.25; length of forewing: 4.95; length of petiole: 0.47; length of gaster: 2.57. Head: in frontal view, eyes longer than the distance between them dorsally; median ocellus wider than the distance between a lateral ocellus and the margin of the eye; clypeus in profile not strongly protuberent. Mesosoma: pronotum, mesopleural area above and below episternal suture feebly shining, not striate; mesonotum finely striate, transversely on anterior quarter, remainder longitudinally; scutellum convex, shining, lacking a median carina; propodeum without a raised carina, feebly shining, with weak, fine striae in the following patterns: a median series of concentric ovals, posterior portion with transverse bisinuate lines, obliquely longitudinal lines laterally ventral to spiracle; propodeum in profile long and low, without a declivitous posterior portion; gaster shining, tergites without surface sculpture except for fine, hair-bearing punctures, evenly covered with long, fine, sub-appressed hairs.

Type localities and associated information, as appear on specimen labels.—Holotype Worker. FL: Highlands Co., Archbold Biological Station, 15-IV-1996, M. Deyrup, recently burned mature scrub, Red Hill, nest in sand. Paratype dealate queen used for description of queen (designated on label). Same locality, collector as holotype, 18-I-1983, Florida scrub habitat with *Ceratiola ericoides*, E. side of Tract 7. Paratype male used for description of male (designated on label). Same locality, collector as holotype, 8-XI-1990, at window, main building. Paratype workers and queens (all from Florida). The following specimens all have the same locality and collector as the holotype: 18 workers: same data as holotype (nest series with holotype); 2 workers: 30-IX-1982, sandhill area with *Quercus laevis*, SE Tract, at bait; 4 workers: 3-X-1982, in patch of *Hypericum*, road 19E, at bait; 2 workers: 19-IX-1982, mature stand of *Pinus clausa*, SE Tract, trail 10, at bait; 3 workers: 30-XIII-1985, mature sand pine scrub habitat, Red Hill, trail 1; 1 worker: 28-X-1982; 1 worker: 7-VI-1984, sandhill habitat, Tract 19E; 1 dealate queen with associated worker: 13-X-1982, in leaf litter of *Bejaria racemosa*, Tract 7, road 18; 1 worker: 24-XI-1982, in dry leaf litter at base of oak, sandhill habitat, Tract 19; 2 workers: 28-VI-1985, in leaf litter of *Carya floridana*, sandhill habitat, Tract 19E; 1 worker: 6-IV-1983, mature *Pinus clausa* scrub, in pan trap below Townes trap; 1 worker: 5-IX-1985, mature *Pinus clausa* scrub, NE firelane of NE Tract; 1 worker: 14-XI-1985, mature sand pine scrub, Red Hill, SE Tract; 1 worker: 13-VI-1985, sand pine scrub habitat; 1 worker: 17-X-1988; 1 worker: 3-X-1982, in tussock of *Andropogon*; 1 worker: 6-VII-1984, mature sand pine scrub habitat, NE firelane of NE Tract; 1 worker: 25-VII-1984, mature sand pine scrub habitat, NE firelane of NE Tract; 1 worker: 28-VI-1985, Tract 19E, sandhill habitat. Paratype workers and queens not from holotype locality: 3 workers: Highlands Co., Lake Placid, 18-IX-1987, P. Martin, sand pine scrub habitat in former YMCA Camp 2 mi. S. of town on Highway US 27; 1 worker: same locality, collector, habitat as previ-

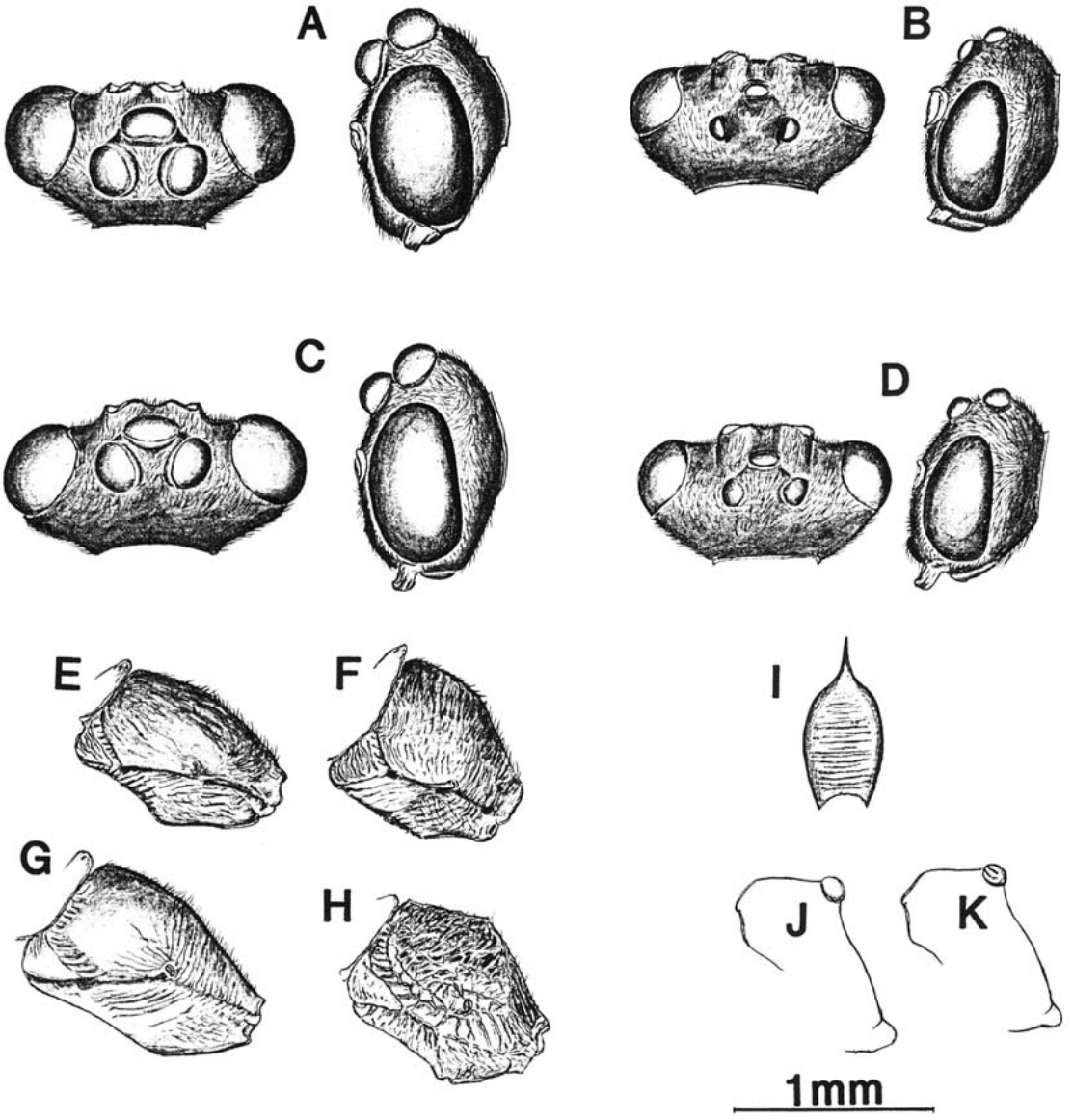

Fig. 2. *Odontomachus* species. A-D: heads of males, occipital and lateral views; A: *relictus*, B: *clarus*, C: *brunneus*, D: *ruginodis*. E-H: propodeal areas of males, lateral views; E: *relictus*, F: *clarus*, G: *brunneus*, H. *ruginodis*. At various times *relictus* (A, E) has been confused with *clarus* (B, F) and *brunneus* (C, G) with *ruginodis* (D, H). I: *ruginodis*, posterior side of petiole of worker. J-K: basalar sclerites (oval structure at upper right corner of mesopleuron) of workers: J: *clarus*, K: *relictus*.

ous, 30-X-1987; 3 workers: same locality, habitat as previous, 27-X-1987, J. Cronin, 1 worker: Highlands Co., Lake Placid, 15-X-1986, M. Deyrup, Holmes Avenue scrub site east of town; 3 workers: Highlands Co., Sebring, 11-IX-1987, P. Martin, former Flamingo Villas Devel., 3.7 mi. SE of Sebring, sand pine scrub; 2 workers: same locality, habitat as previous, 10-XI-1987, J. Cronin; 1 dealate queen with associated worker: same locality, habitat as previous, 17-IX-1990, M. Deyrup; 1 worker: Polk Co., TNC Tiger Creek Preserve, 5-X-1989, M. Deyrup, recently burned scrub with *Quercus laevis*; 1 worker: Polk Co., Lake Wales, 26-I-1988, P. Martin, Flaming Arrow Boy Scout Camp, east of town, sand pine scrub; 1 dealate queen: same site, habitat as previous, 24-XI-1987, J. Cronin; 12 workers: Orange Co., Walt Disney World, S16, T24S, R27E, 5-VII-1996, Z. Prusak, sand pine/oak scrub zone MW-5 (unburned) #88; 2 workers: Orange Co., Walt Disney World, 22-VII-1996, Z. Prusak, MW-5 Cons. Area, unburned zone, sand pine scrub; 2 dealate queens, 4 work-

ers: Citrus Co., 12 mi. NW of Brooksville, 11-V-2002, M. Deyrup & J. Mosley, Withlacoochee State Forest, Sugar Mill Tract, north of 480, sandhill with oaks; 4 workers: Citrus Co., 5 mi. W. of Inverness, 14-IX-1991, M. Deyrup, sandhill habitat, Withlacoochee State Forest; 2 workers: same locality, collector, habitat as previous, 14-XI-1991; 4 workers: Citrus Co., Withlacoochee State Forest, 14-XI-1991, M. Deyrup, 3.2 km south on forest road that begins 7.2 km west of jct. State Road 44 and County Road 581, open sandhill habitat, sparse ground cover of *Aristida beyrichiana*, scattered mature *Pinus palustris* and *Quercus laevis*; 5 workers: Citrus Co., Pine-Oak Estates, 1-IV-1993, M. Deyrup, sandhill habitat along Rt. 488, 3 mi. NE jct. with Rt. 495. Paratype males. The following males are from the Archbold Biological Station, collected by M. Deyrup in Townes flight traps on trails through mature sand pine scrub; dates as follows: 2: 19-VI-1985; 1: 8-VII-1985; 2: 29-VI-1985; 1: 13-VII-1985; 1: 14-VII-1985; 1: 10-VI-1985; 1: 26-VI-1985; 2: 5-VII-1985; 4: 1-VII-1985; 1: 9-X-1987; 1: 28-XII-1983; 1: 16-VII-1986; 1: 24-VII-1985; 1: 21-VII-1983; 1: 3-VII-1983; 1: 27-VI-1983; 2: 28-VI-1983; 1: 21-VI-1983; 1: 20-XI-1985; 1: 8-VII-1983; 1: 23-VIII-1984. Additional paratype males: 7: Orange Co., Walt Disney World, S16, T24S, R27E, 1-5-VII-1996, Z. Prusak, sand pine/oak scrub zone MW-5 (unburned), Malaise trap; 2: Orange Co., Walt Disney World, 8-13-VIII-1996, Z. Prusak, MW-5 Cons. Area unburned zone, sand pine scrub, Malaise trap.

Deposition of types

Holotype, 3 paratype dealate queens, 32 paratype workers, 12 paratype males: Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; 6 paratype workers, 4 paratype males: The Natural History Museum, London; 7 paratype workers, 4 paratype males: Los Angeles County Museum, Los Angeles, California; 10 paratype workers, 4 paratype males: Florida State Collection of Arthropods, Gainesville, Florida; remaining type material in the arthropod collection of the Archbold Biological Station, Lake Placid, Florida.

Etymology

relictus, past participle of *relinquo*: left behind, referring to the distribution of the species on relict patches of Florida scrub and sandhill vegetation on high sand ridges in south-central Florida.

Relationship between *relictus* and other species

Workers of *relictus* and *clarus* are morphologically similar, except for the striate basalar lobe (Fig. 2K) and consistently spinose petiole of *relictus*. Until we compared the males of the two species, we had interpreted these differences as small divergences between widely separated populations of a single species, especially since *clarus* shows considerable variation through the Southwest. The differences between males are the only clear evidence at present that *clarus* and *relictus* are distinct. The extraordinarily large eyes and ocelli of *relictus* (Fig. 2A) suggest that there is some feature of *relictus* flight behavior that is different from the flight behavior of *clarus*; any major difference may be likely to confer reproductive isolation. A possibly relevant feature of *relictus* flight behavior is that male activity, as monitored by Malaise traps, seems to be concentrated around moonlit nights (Deyrup et al. 1985). Another major structural difference is the longer, less declivitous propodeum of *relictus*; all four species occurring in the U.S. show conspicuous differences in the shape and sculpture of the propodeal area (Figs. 2E-H).

It is tempting to hypothesize that *relictus* is closely related to *clarus*, representing an eastern offshoot of a western lineage adapted to dry habitats. Some other animals, such as the Florida sand roach (*Arenivaga floridensis* Caudell), the Florida scrub-jay (*Aphelocoma coerulescens*) and the gopher tortoise (*Gopherus polyphemus*) seem to be examples of western lineages in relic desert-like habitats in Florida (Deyrup 1989). These animals could have spread east, along with a rich fauna of now extinct savanna-dwelling vertebrates, along an arid corridor in southern North America during the late Pliocene through mid-Pleistocene (Webb 1990). In Deyrup's 1989 and 1990 papers *relictus*, under the name of *clarus*, is specifically mentioned as an example of such a western lineage. Male *relictus*, however, do not support this hypothesis. They share their large eyes and ocelli, non-carinate scutellum and low propodeum with the other native southeastern species, *brunneus* (Figs. 2C, G). When the taxonomic status and distribution of southeastern *brunneus* is better understood, it may be possible to propose a new hypothesis on the derivation of *relictus*.

All four of the U.S. species of *Odontomachus* are combined with 17 New World species and two Old World species in the *haematodus* species group, distinguished by the reduction of the segments of the labial palps from four to three (Brown 1976).

Habitat and distribution of *relictus*

This species is a subterranean nester, and found only in areas of deep, unconsolidated, silica sand. These areas may be covered with Florida scrub vegetation, consisting of scattered pines, small oaks and other small trees and shrubs. Sometimes there are areas of bare sand, especially in the first few years following a fire. Alternatively, areas where *relictus* occurs may be sandhill vegetation, consisting of scattered pines

above a low layer of grasses and forbs. For descriptions of these habitats, see Myers (1990). Nest entrances are not marked by a mound, but by scattered pellets of sand. Digging into a nest may produce a few workers, sometimes with brood, but no large aggregation of workers.

Odontomachus relictus is known from the Lake Wales Ridge, the southern Brooksville Ridge and the Orlando Ridge. It has not been found in scrub or sandhill habitats on the Atlantic Coastal Ridge, the Northern Brooksville Ridge, or the sandy uplands of northern Florida. The inland south-central sand ridges of the Florida Peninsula are over a million years old, and are known to have many plants and animals found nowhere else (Deyrup 1989, 1990). Some of these species appear to be remnant populations of species that were once more widespread, others are probably true (autochthonous) endemics. Up to the discovery of the distinct status of *O. relictus*, it seemed that this ant was an example of a series of remnant populations; now it appears that this species could just as easily be a true endemic of south-central Florida.

The restricted range and habitat of *O. relictus* might raise questions about its conservation status. About twenty-five years ago, its prospects would have seemed poor. At that time there were only two protected populations, one on the Lake Wales Ridge (Archbold Biological Station), the other on the southern Brooksville Ridge (Withlacoochee State Forest). Upland areas were being converted rapidly to housing and agriculture, and it seemed that few scrub and sandhill areas would remain within the range of *O. relictus*. Since that time, development and habitat destruction have occurred at an unprecedented rate, but the establishment of ecological preserves also has been remarkably fast, especially on the Lake Wales Ridge. This species now appears to be adequately protected, unless it is subjected to some widespread environmental threat that pervades natural areas. *Odontomachus relictus* is a good example of a species that seemed destined for the endangered species list, with all the trouble and expense implied in such listing, but was preemptively protected by more general conservation programs.

Species of *Odontomachus* in the U.S.

The following keys distinguish between the four species known from the U.S. Brief comments on distribution, nomenclature and natural history follow the keys.

Key to Worker and Queen *Odontomachus* of the U.S.

1. Hairs on first gastral tergite extremely fine and dense: spaces between hairs less than one-third as wide as the length of hairs (SE U.S., perhaps Neotropics) . . . . . *brunneus* (Patton)

1'. Hairs on first gastral tergite sparse, spaces between hairs at least one half as wide as length of hairs 2

2. Posterior face of petiole with conspicuous transverse striae (Fig. 2I) (Florida, W. Indies, perhaps elsewhere in the Neotropics) . . . . . *ruginodis* M. R. Smith

2'. Posterior face of petiole smooth (as in Fig. 1C) 3

3. Basalar lobe (oval sclerite at posterior dorsal corner of mesopleuron) conspicuously striate (Fig. 2K) (south-central peninsular Florida) . . . . . *relictus*, new species

3'. Basalar lobe smooth (Fig. 2J) (southwestern U.S., Mexico) . . . . . *clarus* Roger

Key to Male *Odontomachus* of the U.S.

1. Each ocellus at least as wide as space between lateral ocelli and eye (Figs. 2A, C) 2

1'. Ocelli much smaller than space between lateral ocelli and eye (Figs. 2B, D) 3

2. Head and body pale orange, antennae brown . . . . . *brunneus*

2'. Head and body brown, antennae yellowish . . . . . *relictus*

3. Head and body mostly yellowish, propodeum and sometimes gaster contrasting brown; central area of pronotum smooth and shining . . . . . *ruginodis*

3'. Head and body dark brown; pronotum finely striate . . . . . *clarus*

Notes on Species

Odontomachus brunneus. This species apparently occurs throughout Florida, although there are no records from the three westernmost counties. It is also known from southern Georgia and Alabama. We have seen specimens from low coastal areas in Alabama, and there is no obvious reason why it should not occur in coastal Mississippi, Louisiana and Texas, although it is not re-

ported from any of those states. A report of *brunneus* from Cuba (Fontenla 1997) might refer to some other species, as we have seen specimens that would key to *brunneus* from the Dominican Republic, but are probably closer to *O. insularis* Guerin. Its distribution in Central and South America is unclear, since this species was combined with *O. ruginodis* in Brown's revision of the genus (1976). Associating the various *brunneus*-like forms with their males in Central and South America and the West Indies would be an interesting and useful project for local myrmecologists, and might easily yield distributional surprises or new species. Workers can easily be distinguished from other U.S. species by the densely hairy gaster.

Southeastern records of *insularis* in the Formicidae section of the Catalog of the Hymenoptera (D. R. Smith 1979) refer to *brunneus*. Although the catalog appeared several years after Brown's revision, the cut-off date for changes in the Formicidae section was mid-1975. Smith's treatment of North American *Odontomachus* differs from those of Creighton (1950) and M. R. Smith (1951) in elevating to species level three subspecies of *O. haematodus* (Linnaeus). This was backed by no taxonomic references, and certainly was not intended to compete with the earlier, but unavailable, revision by Brown.

Odontomachus brunneus occurs in both well-drained and poorly drained habitats; nests may be in soil or in rotten wood. This species, along with many others, was studied by Van Pelt (1958) at the Welaka Reserve (now Welaka State Forest) in Putnam Co., Florida. Van Pelt found many colonies, which occurred in all the terrestrial habitats in the area, including flatwoods, mesic forest, swamp forest, upland scrub and sandhill. Nests were in various microhabitats, including deep leaf litter, fallen logs, at the bases of trees, and open or sparsely covered sandy areas. At the Archbold Biological Station, *brunneus* occurs in moist habitats, including flatwoods, bayheads, the edges of seasonal ponds, and elevated tussocks or fallen pines within seasonal ponds. It has not been found in the more elevated upland areas of the Station, which are occupied by *relictus*. This distribution gives the impression that there is some competitive displacement based on differential adaptation to moisture conditions, but the evidence remains circumstantial. It may be relevant that in parts of its range devoid of *relictus*, where *brunneus* occurs in dry, upland areas individuals never achieve the large size and dark color seen in some specimens from wet areas. Nobody knows, however, whether the smaller, paler individuals represent stressed individuals in suboptimal conditions, or whether they represent an adaptive phenotypic response in a robust population.

Workers of *brunneus* sometimes emerge to forage on cloudy days, but are generally nocturnal. The formidable jaws of *brunneus* are not used as assertively as one might expect, and there is fragmentary evidence that *brunneus* is sensitive to chemical defenses. Prey are approached tentatively, and the ant recoils immediately after striking the prey (Brown 1976). There may be a delay before the prey is picked up and carried away; Brown (1976) suggested that these ants react to chemical defenses, which are allowed to dissipate before the prey is retrieved. Alex Wild, while a student at the Archbold Biological Station, twice observed *brunneus* retreating hastily when confronted by aroused workers of *Dorymyrmex bureni* (Trager) (unpublished natural history notes on file at the Archbold Biological Station). *Dorymyrmex bureni* is much smaller than *O. brunneus*, but can release large quantities of defensive chemicals that are pungent to the human nose. Van Pelt (1958) reported accumulations of *brunneus* head capsules in the nests of *Formica archboldi* Smith, and suggested the possibility that *brunneus* is a regular part of the diet of *F. archboldi*. If this is the case, it is more likely that the *brunneus* are subdued by chemical means than by mandible-to-mandible combat.

During this study specimens were examined from the following areas: FL: Alachua, Baker, Bay, Bradford, Brevard, Broward, Citrus, Clay, Collier, Columbia, Dade, De Soto, Dixie, Duval, Franklin, Gadsden, Gilchrist, Glades, Hamilton, Hernando, Highlands, Hillsborough, Indian River, Jackson, Jefferson, Lake, Lee, Leon, Levy, Liberty, Madison, Marion, Martin, Monroe, Nassau, Okeechobee, Orange, Osceola, Palm Beach, Pasco, Polk, Putnam, Sarasota, St. Lucie, Sumter, Taylor, Volusia, Wakulla, Walton Counties; GA: Clinch County; AL: Baldwin, Houston Counties.

Odontomachus clarus. This species is known from northern Mexico and from Texas, New Mexico and Arizona. It is the only *Odontomachus* known from the southwestern U.S. and northern border of Mexico, so specimens may be identified tentatively by their source alone. The similar species *ruginodis* will probably be transported to the southwestern U.S.; *ruginodis* has conspicuous striae on the posterior face of the petiole (Fig. 2I). It is also possible that the species we consider *clarus* includes unrecognized cryptic species. If this is the case, the occurrence of additional species might be detected by finding more than one form of male within the range of *clarus*. We have seen males associated with workers of *clarus* from two widely separated sites: Cochise Co., AZ, and Jeff Davis Co., TX. In our experience, it is difficult to find workers of *clarus* with associated males. Examination of specimens from light traps or flight traps might be a convenient way to establish whether there is more than one western species. Records of *clarus* from the West Indies (Smith 1979) refer to some other species, perhaps *ruginodis*. All references to *clarus* in Florida (Deyrup et al. 1985; Deyrup 1989; Deyrup et al.

1989; Deyrup 1990; Sivinski et al. 1998) should be referred to *relictus*, as discussed above.

The species names *coninodis* Wheeler and *desertorum* Wheeler, listed in the 1979 catalog, were synonymized under *clarus* by Brown (1976). Brown reported that "*coninodis*," which has a blunt petiolar spine, occurs at the higher elevations in Arizona, in isolated, low mountain ranges, surrounded by lower areas occupied by typical *clarus* with an elongate petiolar spine. The distribution of the two forms is unlike that of a normal pair of geographic subspecies, and Brown characterized the short-spined forms as "depauperate ecotypes or ecophenotypes." As in the case (mentioned above) of the smaller, paler *brunneus* found in dry sites, it seems premature to apply the pejorative "depauperate" to a condition that, for all we know, could be a superb adaptive response.

In Arizona this species is found, usually in small numbers, under rocks and grass tussocks, in both dry and mesic sites. In western Texas it shows a preference for more mesic sites and fine soils; nests are usually found under rocks or logs (Cokendolpher & Francke1990).

During this study specimens were examined from the following sites (we provide more detailed site information for *clarus* than for *brunneus* or *ruginodis* because of evidence of geographic variability in *clarus*). AZ: Cochise Co. (Chiricahua Mts: Cave Creek, Texas, and Idlewild Canyons), Pima Co. (Tucson), Santa Cruz Co. (Patagonia Mts., Pajarito Mts.); TX: Bosque Co. (Meridian), Brewster Co. (Big Bend National Park: Rio Grande Village), Denton Co. (no locality), Jeff Davis Co. (Davis Mts.), Travis Co. (Bull Creek, McNeil); MEXICO: Chihuahua (Riva Palacio, Guerero, Conchos), Coahuilla (25 km E. of Saltillo), Cuernovaca (no locality), Guanjuato (highway 57 km 57), Hidalgo (San Miguel), Jalisco (Guadelajara), Mexico (Pedrigales), Nuevo Leon ((Monterrey), Queretaro (3 mi. W. of Queretaro).

Odontomachus ruginodis. This species occurs sporadically through southern and central Florida, at least as far north as Orlando, and also in the West Indies. Its distribution in South and Central America is unclear because it has been confused with *brunneus*. Its ability to thrive in disturbed habitats should allow it to invade mainland Neotropical areas, if it is not already present. It is probable that this species will be distributed by commerce to disturbed areas in the Southwest. The conspicuous striae on the posterior face of the petiole (Fig. 2I) distinguish workers of this species from the similar *clarus* and *relictus*, but there are additional species with petiolar striae (e.g., *O. bauri* Emery) outside the U.S.

The name *ruginodis* was first applied by Wheeler (1905), and Wheeler was designated as the author of *ruginodis* in Deyrup et al. (1985) and Deyrup et al. (1989). The name *ruginodis*, however, was first used as a quadrinomial (*Odontomachus haematodus insularis ruginodis*), and therefore is not a valid name under the rules of nomenclature. The first use of *ruginodis* as a trinomial, or subspecies (*Odontomachus haematodus ruginodis*), was by M. R. Smith (1937). Since this is the first valid use of *ruginodis* for this species, M. R. Smith is the author of the name. This, along with hundreds of other tangles of nomenclature, was straightened out in Bolton's 1995 catalog of ants.

In Florida, this species occurs in disturbed areas, including urban and suburban habitats. It occurs along the beaches in the tropical part of the state. It has not yet been found inland in natural habitats. In Puerto Rico it differs from another sympatric species (perhaps *O. bauri*) in its preference for open, sunny areas, especially river bottoms (Smith 1937).

The defensive mandible-snapping behavior of *ruginodis* was studied by Carlin and Gladstein (1989). When a nest is attacked by other ants, the *ruginodis* workers rush out, snapping at anything that seems a threat. Enemy ants may be dismembered or knocked out of the way by the mandibular strikes. If the mandibles hit a solid object, the *ruginodis* may itself be flung into the air for a distance of several centimeters. This does not seem to be an escape mechanism, as the worker, upon landing, immediately charges back into the fray. The nest entrance is usually guarded by a single worker, who stands with cocked mandibles near the entrance. If an intruder approaches within striking distance, the mandibles snap shut, responding to signals from the antennae and long sensory hairs at the bases of the mandibles. The heavy apices of the mandibles do not slice into the intruder, but knock it away a distance of about one to fourteen centimeters. Carlin and Gladstein call this the "bouncer defense."

During this study specimens were examined from the following areas: FL: Brevard, Broward, Charlotte, Collier, Dade, Glades, Hendry, Highlands, Hillsborough, Indian River, Lee, Manatee, Martin, Monroe, Orange, Palm Beach, Pinellas, Orange, Polk, St. Lucie, Volusia Counties; BERMUDA; BAHAMAS: New Providence, San Salvador, Rum Cay, North Andros Islands; PUERTO RICO: Rio Grande.

Residual Problems

There are still some questions on the taxonomy and distribution of the *Odontomachus* species that occur in the U.S. The distribution of *brunneus* and *ruginodis* is unclear. Does *brunneus* occur in coastal wetlands around the Gulf of Mexico? Is *brunneus* as it appears in the southeastern North America the same species as the *brunneus* populations reported from the West Indies and the mainland Neotropics? Does *rugino-*

dis occur outside the West Indies and Florida? Another kind of question deals with the morphological divergences between males of different species. Do the kind of differences we have reported relate to differences in ecology and behavior of the species? Will *Odontomachus* males prove useful in distinguishing species throughout the Neotropics? The few males of *insularis* and *bauri* that we have seen show conspicuous species differences, but there could be species complexes that cannot be elucidated by male morphology.

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MOLECULAR DIAGNOSTICS OF THE FORMOSAN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE)

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ABSTRACT

Formosan subterranean termite, *Coptotermes formosanus* Shriaki, is a serious pest of structures in portions of United States. A 467-bp region of the mtDNA 16S rRNA gene was subjected to DNA sequencing from 12 *Coptotermes* species, including 64 populations of *C. formosanus*. Genetic diversity among species ranged from 1.8% to 7.0%, with *C. formosanus* at least 3.0% divergent to the other *Coptotermes* taxa. No genetic variation was detected among the *C. formosanus* populations for this marker making it ideal for diagnostics. Comparison of nucleotide sequence of mitochondrial rRNA 16S was used to design polymerase chain reaction (PCR) primers specific for *C. formosanus*. The diagnostic assay consists of two independent PCR runs of the 16S primer pair along with the *C. formosanus* primer set. PCR product from samples that are not *C. formosanus* can be subjected to DNA sequencing and compared with the database of termite 16S sequences on GenBank for identification. This technique provides a non-morphological method to identify field collected termites and may facilitate future quarantine programs for *C. formosanus*.

Key Words: *Coptotermes formosanus*, invasive species, PCR, genetic variation, molecular diagnostics, termite.

RESUMEN

La termita subterráneo de Formosa, *Coptotermes formosanus* Shriaki, es una plaga seria de las estructuras en algunas partes de los Estados Unidos. Una región de 467 bp del gen 16S rARN de la ADN mitocondrial fue sujetada de la secuenciación de AND de 12 especies de *Coptotermes*, incluyendo 64 poblaciones de *C. formosanus*. La diversidad genética entre las especies fue de 1.8% hasta 7.0%, con la *C. formosanus* por lo menos 3.0% divergentes de los otros especies de *Coptotermes*. Ningún variación genética fue detectada entre las poblaciones de *C. formosanus* para este marcador haciendole ideal para un diagnóstico. Una comparición de la secuencia del nucleótido del gen 16S rARN de la ADN mitocondrial fue usada para diseñar unos cebadores (primers) específicos de la reacción en cadena por la polimerasa (PCR) para *C. formosanus*. El ensayo diagnóstico consiste de dos pruebas independientes de PCR del par 16S del cebador junto con el grupo de cebadores de *C. formosanus.* El producto de PCR de las muestras que no son *C. formosanus* puede ser sujetado de la secuenciación de ADN y comparados con el base de datos de las secuencias de 16S de termitas en el GenBank para la identificación. Esta técnica provea un método no morfológico para identificar las termitas recolectadas en el campo y puede facilitar los futuros programas de cuarentena para *C. formosanus*.

The Formosan subterranean termite (FST) *Coptotermes formosanus* Shriaki (Isoptera: Rhinotermitidae), is a major economic pest worldwide and has become a serious pest to the United States and its territories. Native to China, FST has been introduced into Japan, Guam, Sri Lanka, South Africa, Hawaii and the continental United States (Mori 1987; Su & Tamashiro 1987; Wang & Grace 1999). *Coptotermes formosanus* was first recorded in continental United States at Charleston, SC in 1957 (Chambers et al. 1988). Numerous well-established colonies were discovered in Florida in 1980, 1982, and 1984 (Oi et al. 1992) with many additional finds since (Su & Scheffrahn 2000). Introductions to San Diego, CA (Atkinson et al. 1993; Haagsma et al. 1995), the Gulf Coast states, and southeastern US also have been documented (Spink 1967; LaFage 1987; Su & Tamashiro 1987; Howell et al. 1987; Appel & Sponsler 1989; Oi et al. 1992; Su & Scheffrahn 1998; Hawthorne et al. 2000; Howell et al. 2000, Scheffrahn et al. 2001; Hu et al. 2001; Jenkins et al. 2002). Since 2002, *C. formosanus* has been considered a quarantine pest in Mississippi (Missis-

sippi Department of Agriculture and Commerce, Rule 40). In the city of New Orleans, the control and repair costs due to FST are estimated at $300 million annually (Lax & Osbrink 2003) and annual damage to the entire United States is estimated to exceed $1 billion. It is considered the single most economically important insect pest in the state of Hawaii (Su & Tamashiro 1987).

The inability to quickly discriminate what *Coptotermes* species one is dealing with could lead to difficulties in evaluating the source of the infestation. While introductions of FST have gained recent notoriety, less is reported or known about other potentially damaging *Coptotermes* species. Exotic introductions of *Coptotermes havilandi* to Florida (Su et al. 1997; Su et al. 2000; Scheffrahn & Su 2000) have been detected. Although not established, *C. havilandi* Holmgren, which recently has been synonymized as *C. gestroi* (Wasmann) by Kirton & Brown (2003), has been recovered in shipping crates in Tennessee imported from East Asia (RHS, unpublished data). *Coptotermes* from South America, Central America, and the Caribbean also pose potential problems for the US. There are three described endemic species of *Coptotermes* in the Americas including *C. crassus* Snyder, *C. testaceus* L., and *C. niger* Snyder, and possibly others which have not been identified. More recent genetic surveys have uncovered old world *Coptotermes* species, *C. sojesti*, introduced to the West Indies (Scheffrahn et al. in press).

Mistakes have been made in both the naming and correct identification of some *Coptotermes*. For example, the inconsistencies in the pest status of *C. havilandi* in different regions of its geographic range have been due to misidentification and taxonomic confusion between *C. travians* (Haviland), *C. havilandi*, and *C. gestroi* (Kirton & Brown 2003). An examination of the type series of *C. travians* indicates the species has been misidentified in Peninsular Malaysian literature (Tho 1992) as *C. havilandi* and also has been referred to as *C. borneensis* Oshima (Kirton & Brown 2003).

Correct identification is critical for pest insects. Identification of termite workers is possible at the generic level only, and finding an alate, which can be identified, in a collection is seasonal and can be rare. We have developed a molecular diagnostic method capable of differentiating FST from other *Coptotermes* species regardless of the caste encountered or locality obtained. It has been demonstrated that both nucleotide sequencing and restriction enzyme digestion of polymerase chain reaction (PCR) amplicons can be used to differentiate between various termite species (Austin et al. 2002; Austin et al. 2004; Szalanski et al. 2003; Clement et al. 2001; Jenkins et al. 2002; Uva et al. 2004). DNA sequence differences reported between *Coptotermes* species from numerous disjunctive populations from around the world in a small portion of the mtDNA were exploited to design species-specific PCR primers and to develop a DNA-based assay that can discriminate FST from other *Coptotermes* species.

Materials and Methods

Coptotermes termites were collected from various locations in North America, South America, the Caribbean, Australia, Africa, and Asia (Table 1). Morphological identification of specimens used in this study was achieved by using Snyder (1922), Emerson (1925, 1928), Hill (1942), Scheffrahn et al. (1990), Scheffrahn & Su (1994), Tho (1992), and Su et al. (1997). Voucher specimens, preserved in 100% ethanol, are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR, USA.

Alcohol preserved specimens were allowed to dry on filter paper, and DNA was extracted from individual worker or soldier heads with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 µl of Tris:EDTA and stored at -20°C. Polymerase chain reaction was conducted with the primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al., 1994). These PCR primers amplify an approximately 428-bp region of the mtDNA 16S rRNA gene. PCR reactions were conducted with 1 µl of the extracted DNA per Szalanski et al. (2000), and a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 45 s. Amplified DNA from individual termites was purified and concentrated by Microcon-PCR Filter Units (Millipore, Bedford, MA). Samples were sent to The University of Arkansas Medical School DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions with an ABI Prism 377 DNA sequencer (Foster City, CA). GenBank accession numbers for the *Coptotermes* termites subjected to DNA sequencing in this study are AY558898 to AY558914. DNA sequences were aligned by the PILEUP command of GCG (Accelrys, San Diego, CA), and the distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic diversity according to the Kimura 2-parameter model (Kimura 1980) of sequence evolution.

Results and Discussion

The 428-bp region of the mtDNA 16S rRNA gene was subjected to DNA sequencing from 12 described species of *Coptotermes*, including 64 populations of *C. formosanus* (Table 1). Within the genus, genetic diversity ranged from 1.8% between *C. testaceus* and *C. crassus* to 7.0% between *C. acinaciformis* and *C. vastator*. No genetic variation was observed between the two *C. testaceus* samples, and up to 0.7% variation was observed among the *C. gestroi* samples. No genetic varia-

TABLE 1. *COPTOTERMES* COLLECTION DATA.

Species	Location	Country	N[a]
C. acinaciformis		Australia	1
C. carvinatus		Malaysia	1
C. crassus	Belize City	Belize	1
C. formosanus	San Diego, CA	USA	1
	Jacksonville, FL	USA	1
	Wilton Manors, FL	USA	1
	Stone Mt., GA	USA	1
	Oahu, HI	USA	2
	Maui, HI	USA	2
	Lake Charles, LA	USA	8
	New Orleans, LA	USA	1
	Baton Rouge, LA	USA	1
	St. Rose, LA	USA	1
	Stennis Space Ctr, MS	USA	7
	Spindale, NC	USA	1
	Forest City, NC	USA	1
	Rutherfordton, NC	USA	1
	Rockport, TX	USA	1
	Rockwall, TX	USA	1
	Galveston, TX	USA	8
	Garland, TX	USA	7
	Grapevine, TX	USA	2
	Lewisville, TX	USA	1
	San Antonio, TX	USA	1
	Beaumont, TX	USA	1
	La Porte, TX	USA	1
	Hong Kong	China	8
	Guangzhou	China	1
		Taiwan	1
	Nagasaki Prefecture	Japan	2
C. heimi		India	1
C. gestroi		Grand Turk	1
	Monroe, FL	USA	1
	Miami, FL	USA	1
		Singapore	1
		Taiwan	1
	Hong Kong	China	1
C. lacteus	Berrburrum	Australia	2
C. intermedius	Togo	Africa	1
C. michaelseni	Perth	Australia	4
C. sjostedti	Nongo	Guinea	1
C. testaceus		Tobago	1
		Grenada	1
C. vasator	Oahu, HI	USA	1

[a]Number sequenced.

tion was observed in *C. formosanus*, and *C. formosanus* was most similar to the *C. intermedius* sample from Togo Africa, with 3.0% DNA sequence divergence. Phylogenetically, *C. formosanus* forms a distinct clade among non-Australian *Coptotermes* (*C. vastator*, *C. testaceus*, *C. crassus*, *C. sjostedti*, *C. intermedius*, *C. gestroi*, *C. heimi*, and *C. carvinatus*) (Scheffrahn et al. in press).

Formosan subterranean termite 16S DNA sequences along with sequences from other *Coptotermes*, *Reticulitermes* (Szalanski et al. 2003) and *Heterotermes* (Szalanski et al. 2004) were aligned and examined for mismatches that reflected either substitutions or deletions. The mismatches were exploited to design primers that were unique to FST (Table 2). Two primers, one from each strand FST-F (5'-TAAAACAAACAAACAACAAACAAAC-3') and FST-R (5'-ATGGCTTGACGAGGCACAA-3') were designed. Based on the sequence, the expected sizes of the amplicon is

TABLE 2. DNA SEQUENCE ALIGNMENT OF 12 *Coptotermes* SP., AND *C. formosanus* SPECIFIC PCR PRIMERS.

FST-F	TAAAACAAAC	AAACAACAAA	CAAAC			
	10	20	30	40	50	60
C. formonsanus	TAAAACAAAC	AAACAACAAA	CAAACAAATA	AACCAAA-TG	TTAAACTCTA	TAGGGTCTTC
C. vastator	-AAACAAAAC	AAACAACATA	AAA-TAAATA	GGCCAAA-TG	TTAAACTCTA	TAGGGTCTTC
C. testaceus Tobago	-AAACAAAAC	AAACAACATA	AA--TAAATA	AGCCAAA-TG	TCAAACTCTA	TAGGGTCTTC
C. crassus	-AAACAAAAC	AAACAACATA	AA--TAAATA	AGCCAAA-TG	TTAAACTCTA	TAGGGTCTTC
C. sjostedti	-AAACAAAAC	AAACAACAAA	GAAATAAACA	AACCAAA-GG	TAAAACTCTA	TAGGGTCTTC
C. intermedius	-AAACAAAAC	AAACAACAAA	AAAATAAATA	AACCAAA-TG	TTAAACTCTA	TAGGGTCTTC
C. gestroi	--AAATAAAC	AAACAACAAA	CAAGTAAATA	AACCAAA-TG	TCAAACTCTA	TAGGGTCTTC
C. heimi	--AAACAAAC	AAACAACAAA	TAAGCAAATA	AACCAAA-TG	TCAAACTCTA	TAGGGTCTTC
C. carvinatus	CAAAACAAAC	AAACAACCAA	ACAGGAAATA	AACCAAA-TG	TCAAACTCTA	TAGGGTCTTC
C. lacteus	TAAAATAAAC	AAACAAC-AA	CAAATAAGTA	AGCCAAAATG	TCAAACTCTA	TAGGGTCTTC
C. acinaciformis	CAAAATAAAC	AAACAACCAA	CAAATGAATA	AACCAAA-TG	TTAAACTCTA	TAGGGTCTTC
C. michaelseni	TAAATAAAAC	AAACAACCAA	TAAATGAATA	AACTAATATG	TCAAACTCTA	TAGGGTCTTC
	70	80	90	100	110	120
C. formonsanus	TCGTCCCACA	AAAACATCTA	AGAATTTTAA	CTCAAAAACC	AAATTCAATA	AAA-CATTCA
C. vastator	TCGTCCCATA	AAAACATCTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-CATTCA
C. testaceus Tobago	TCGTCCCATA	AAAACATCTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-CATCCA
C. crassus	TCGTCCCATA	AAAACATCTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-CATTCA
C. sjostedti	TCGTCCCACA	AAAACATTTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-CATTCA
C. intermedius	TCGTCCCACA	AAAACATCTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-TATTCA
C. gestroi	TCGTCCCACA	AAAACATCTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-CATTCA
C. heimi	TCGTCCCACA	AAAACATCTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-CATTCA
C. carvinatus	TCGTCCCACA	AAAACATTTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-CATTCA
C. lacteus	TCGTCCCATA	AAAACATTTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-TATCCA
C. acinaciformis	TCGTCCCATA	AAAACATTTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAAATATTCA
C. michaelseni	TCGTCCCATA	AAAACATCTA	AGAATTTTAA	CTCAAAGACC	AAATTCAATA	AAA-TATTCA
		ATGGCTT	GACGAGGCAC	AA FST-R		
	130	140	150			
C. formonsanus	ACATTAAGAC	AGCTTGTGCC	TCGTCAAGCC	AT		
C. vastator	TCACTAAGAC	AGCCAGTGCC	TCGTCAAGCC	AT		
C. testaceus Tobago	TCACTAAGAC	AGCCAGTGCC	TCGTCAAGCC	AT		
C. crassus	TCACTAAGAC	AGCCAGTGCC	TCGTCAAGCC	AT		
C. sjostedti	TCACTAAGAC	AGCCGGTGCC	TCGTCAAGCC	AT		
C. intermedius	TCACTAAGAC	AGCCCGTGCC	TCGTCAAGCC	AT		
C. gestroi	ACACTAAGAC	AGCCCGTGCC	TCGTCAAGCC	AT		
C. heimi	ACACTAAGAC	AGCCCGTGCC	TCGTCAAGCC	AT		
C. carvinatus	ACACTAAGAC	AGCCCGTGCC	TCGTCAAGCC	AT		
C. lacteus	ACACTAAGAC	AGCTCATGTC	TCGTCAAGCC	AT		
C. acinaciformis	ACACTAAGAC	AGCTCATGTC	TCGTCAAGCC	AT		
C. michaelseni	ACACTAAGAC	AGCTCGTGCC	TCGTCAAGCC	AT		

151 bp. Proper sized PCR products were obtained with conspecific DNA, whereas no product was obtained with template from the other species, i.e., no false positives were observed with known DNA. Each FST primer paired with a common primer will only amplify Formosan subterranean termite DNA.

The FST species-specific primers were tested for optimal annealing performance in a 47°-59°C temperature gradient with 2°C intervals. The optimal annealing temperature for the FST specific primers was 57°C. PCR reactions were the same as the 16S conditions with the exception of the PCR profile which consists of 30 cycles of 94°C for 45 s, 57°C for 45 s and 72°C for 45 s. This annealing temperature, however, is too high for the 16S universal primers, preventing multiplex PCR with both primer pairs. To resolve this, both PCR reactions are conducted individually and 10 µl of each PCR reaction are loaded onto a single well of a 2% agarose gel (Fig. 1). The FST specific primer set successfully amplified for 52 individual FST

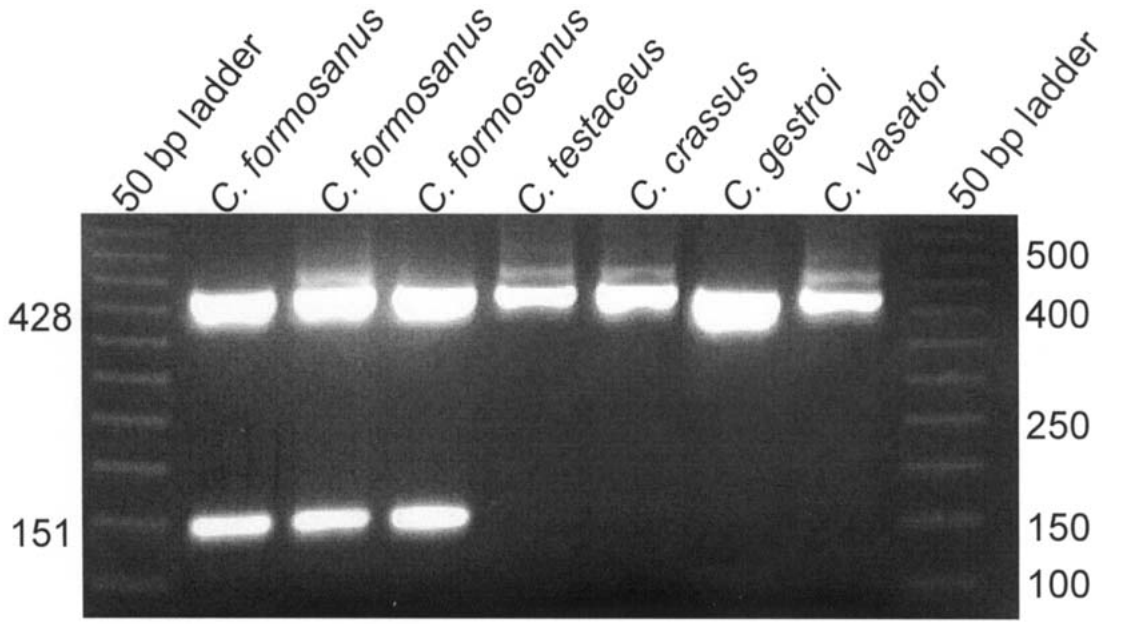

Fig. 1. Two percent agarose gel of 428-bp mtDNA 16S amplicon and 151-bp *C. formosanus* diagnostic amplicon for 4 *Coptotermes* spp.

from all 23 FST populations, and did not yield a PCR product for the other *Coptotermes*, *Reticulitermes* and *Heterotermes*. PCR product from samples that are not *C. formosanus* can be subjected to DNA sequencing and compared with the database of termite 16S sequences on GenBank for identification by a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/). This technique provides a method to identify field collected termites and facilitates the screening of the monitoring for this species and for the introduction of other invasive *Coptotermes* termites.

In the context of determining the species for a large number of samples collected in connection with distribution or competition studies, simplifying the identification of the worker caste is advantageous. FST has no genetic polymorphism across its geographic distribution for the 16S marker, whereas genetic variation has been observed in the mtDNA COII gene (ALS unpublished data, Jenkins et al. 2002). This lack of mtDNA 16S intraspecific variation makes this marker ideal for molecular diagnostics.

These primers provide a convenient way to identify individual termites without resorting to more time consuming restriction fragment-length polymorphism analysis, or extensive morphological data which may result in overlap due to clinal variations in size as observed in many insects including termites. The approach is equally applicable to other castes, such as soldiers and alates, but given their obvious taxonomic importance should be constrained to either morphologically ambiguous samples, or when the more diagnostic castes are unavailable. This should be an important new tool for substantiating the identity of FST before the onset of regulatory procedures (i.e., quarantine).

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DISTRIBUTION AND GENETIC VARIATION OF *RETICULITERMES* (ISOPTERA: RHINOTERMITIDAE) IN OKLAHOMA

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ABSTRACT

Sequencing of a portion of the mitochondrial DNA 16S gene was undertaken to determine genetic variation and distribution of *Reticulitermes* in Oklahoma. From 16 Oklahoma counties, 43 *R. flavipes*, four *R. hageni*, one *R. virginicus*, and seven *R. tibialis* samples were collected, identified and subjected to DNA sequencing. No genetic variation was observed in *R. virginicus*, while two haplotypes were observed in *R. hageni*, four in *R. tibialis*, and 10 for *R. flavipes*. Among the 10 *R. flavipes* haplotypes, nine nucleotides were variable and genetic variation ranged from 0.2 to 1.4%. Phylogenetic analysis revealed several minor relationships within *R. tibialis* and *R. flavipes*; however, there was no apparent geographical association to the haplotypes. The high amount of genetic variation, but a lack of geographically distinct haplotypes in *R. flavipes*, indicate that this termite species has been distributed randomly in Oklahoma by humans due to its association with structures.

Key Words: 16S, DNA sequence, genetic variation, population genetics, *Reticulitermes*, termite.

RESUMEN

Se llevo a cabo la secuenciación del una porción del gen 16S de ADN mitocondrial para determinar la variación genética y distribución de *Reticulitermes* en Oklahoma. De los 16 condados de Oklahoma, 43 *R. flavipes*, cuatro *R. hageni*, un *R. virginicus*, y siete *R. tibialis* muestras fueron recolectadas, identificadas y sujetas a la secuenciación de ADN. Ningún variación genética fue observada en *R. virginicus*, mientras que dos haplotipos fueron observados en *R. hageni*, cuatro en *R. tibialis*, y 10 en *R. flavipes*. Entre los 10 haplotipos de *R. flavipes*, nueve nucleótidos varian y la variación genética fue de 0.2 hasta 1.4%. Un analisis filogenético reveló una relacion menor entre *R. tibialis* y *R. flavipes*; sin embargo, no habia una asociación geográfica aparente entre los haplotipos. La cantidad mas alta de variación genética, junta con la falta de haplotipos distintos geográficos en *R. flavipes*, indica que esta especie de termita ha sida distribuida al azar en Oklahoma por humanos debido a su asociación con estructuras.

Subterranean termites in the genus *Reticulitermes* Holmgren belong to the Isopteran family Rhinotermitidae and contain some of the most destructive and damaging termite species with respect to their wood feeding preference. The four principal subterranean termite species in the United States are the eastern subterranean termite *Reticulitermes flavipes* (Kollar), the arid subterranean termite *R. tibialis* Banks, and the dark-southern subterranean termite *R. virginicus* (Banks). Ninety percent of the termite control business in the United States involves these four *Reticulitermes* species plus *Coptotermes formosanus* (Shiraki) (Forschler & Lewis 1997). In Oklahoma, subterranean termites (Isoptera: Rhinotermitidae) are found throughout the state and cause millions of dollars in structural damage every year. The probability that termites will attack a wooden structure within 10 to 20 years after construction is greater than 70% in Oklahoma (Criswell & Pinkston 2001). While the total economic impact of *Reticulitermes* spp. in Oklahoma is uncertain, anecdotal accounts of their presence and destructive activities within urban areas have been documented (Affeltranger et al. 1987; Anonymous 2001a).

Recently, Brown et al. (2004) conducted a study involving species identification and distribution, and wood consumption rates of termites collected from over 200 sites in Oklahoma using in-ground and surface-ground boards. The most abundant naturally occurring termite species found were in the genus *Reticulitermes*. *Reticulitermes flavipes*, the light-southern subterranean termite *R. hageni* Banks, *R. tibialis*, and *R. virginicus* are known from Oklahoma (Weesner 1965). Presently, the K.C. Emerson Entomology Museum at Oklahoma State University, Stillwater, Oklahoma has 25 *Reticulitermes* specimens of which only 13 have been identified to species (all *Reticulitermes flavipes*). *Reticulitermes* spp. have been reported from less than 25 counties in Oklahoma (out of 75 total) (Anonymous 1999; Anonymous 2000; Anonymous 2001b; Anonymous 2002).

Oklahoma probably has a similar complement of *Reticulitermes* species as found in states with which it has contiguous borders and where surveys have or will be conducted given that they are in the same geographical area with similar climates and habitats with no geographical barriers (Howell et al. 1987; Wang & Powell 2001; Messenger et al. 2002; Austin et al. 2004).

Correctly identifying termites is important because different control methods and strategies may be used depending on the target species. Identifying termite workers to species is difficult, and identifying soldiers is sometimes inaccurate because precise measurements are required and overlap may occur between species (Scheffrahn & Su 1994). Difficulties can arise in species determination from individual collection sites because colonies consist mostly of the worker caste while soldiers are less abundant. Alates are found less frequently in collections given their seasonal occurrences and unpredictable swarming. Soldiers represent only 1-3% of the total population of *Reticulitermes* colonies and are morphologically variable; use of this caste alone for identification can result in equivocal species determinations. Subtle clinal variations imposed by geographic boundaries can influence morphology making correct species determinations difficult.

In contrast, molecular genetic methods are able to differentiate species regardless of caste (Szalanski et al. 2003). Also, genetic information obtained from existing collections can be an integral component to phylogenetic studies as a whole, reflecting potential changes in species distributions over time. The extent of genetic variation and subsequent gene flow in *Reticulitermes* spp. from Oklahoma has never been studied. Previous genetic studies have focused on *Reticulitermes* spp. from the southeastern United States and Western Europe (Jenkins et al. 1998; Jenkins et al. 2001; Marini & Mantovani 2002; Uva et al. 2003). Recently, Austin et al. (2004) conducted the first comprehensive genetic survey of *Reticulitermes* in Texas and found 13 haplotypes of *R. flavipes*, seven *R. tibialis* haplotypes, and one haplotype each for *R. virginicus* and *R. hageni*.

Identification to the species level of specimens from existing collections with molecular techniques as outlined in this study may add significant information on species distribution and gene flow. Genetic variation and gene flow information may elucidate existing patterns of spread, possible hybridization, and general speciation of *Reticulitermes* spp. in Oklahoma. In this study, we investigated the extent of genetic variation within and among Oklahoma *Reticulitermes*, evaluated the utility of genetic markers used for identifying species, expanded the known geographical distribution of these taxa within Oklahoma, and determined if *Reticulitermes* distributions are influenced by human activity.

Materials and Methods

Termites were collected from several locations in Oklahoma (Table 1) and preserved in 100% ethanol. In addition to our own collecting efforts, we solicited the assistance of Pest Management Professionals (PMPs) throughout the state to determine the predominant species found in infested structures. PMPs were provided with collection kits and samples were mailed to our laboratory. Fifty-five samples, representing various geographic zones were used for molecular analysis. When available, *Reticulitermes* alates or soldiers were also morphologically identified to species with keys by Krishna & Weesner (1969), Scheffrahn & Su (1994), Hostettler et al. (1995), Donovan et al. (2000). For samples consisting only of workers, species identification was conducted by using mtDNA 16S sequences (Szalanski et al. 2003). Voucher specimens preserved in 100% ethanol are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

Alcohol-preserved specimens were allowed to dry on filter paper, and DNA was extracted from whole individual termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN) per Austin et al. (2002). Extracted DNA was resuspended in 50 µl of Tris:EDTA and stored at -20°C. Polymerase chain reaction was conducted with primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al. 1994). These PCR primers amplify an approximately 428 bp region of the mtDNA 16S rRNA gene. PCR reactions were conducted with 1 µl of extracted DNA (Szalanski et al. 2000) and a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 60 s. Amplified DNA from individual termites was purified and concentrated with minicolumns (Wizard PCRpreps, Promega Corp., Madison, WI) according to the manufacturer's instructions. Samples were sent to the University of Arkansas DNA Sequencing Facility (Fayetteville, AR) for direct sequencing in both directions. GenBank accession numbers were AY538739 to AY538744 for the termite haplotypes new to this study and not present in Austin et al. (2004). DNA sequences were aligned by the PILEUP command of GCG (Accelrys, San Diego, CA). Mitochondrial DNA haplotypes were aligned by using MacClade v4 (Sinauer Associates, Sunderland, MA).

The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Mitochondrial 16S sequences from the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (GenBank AY558910), and the desert subterranean termite *Heterotermes aureus* (Sny-

TABLE 1. COLLECTION DATA, AND HAPLOTYPES FOR OKLAHOMA *RETICULITERMES* SPP. AND OUTGROUP TAXA.

Species	City	County	Haplotype	N
R. flavipes	Stillwater	Payne	E	1
	—	Payne	E	1
	Spiro	LeFlore	F	1
		McCurtain	F	1
	Norman	Cleveland	F	1
	Stillwater	Payne	F	1
	Grove	Delaware	F	1
	Harrah	Oklahoma	G	1
	Stillwater	Payne	G	1
	Glenpool	Tulsa	H	1
	Mannford	Pawnee	H	1
	Marietta	Love	H	1
	Oklahoma City	Oklahoma	H	1
	Tulsa	Tulsa	H	1
	Tulsa	Tulsa	J	1
	Ardmore	Carter	L	1
	Edmond	Oklahoma	L	2
	Oklahoma City	Oklahoma	L	3
	Slapout	Beaver	L	1
	Stillwater	Payne	L	1
	Tulsa	Tulsa	L	1
	—	McCurtain	L	1
	Oklahoma City	Oklahoma	O	1
	Magnum	Greer	N	1
	—	Greer	N	1
	Colcord	Delaware	P	1
	Edmond	Oklahoma	P	1
	Glenpool	Tulsa	P	1
	Grove	Delaware	P	1
	Mannford	Pawnee	P	1
	Oklahoma City	Oklahoma	P	2
	Owasso	#Tulsa	P	2
	Tulsa	Tulsa	P	4
	—	Wagoner	P	1
	Monkey Island	Ottawa	Q	1
R. hageni	Fort Towson	Choctaw	H1	1
	Grove	Delaware	H1	1
	Jay	Delaware	H2	1
	Fort Towson	Choctaw	H2	1
R. virginicus	Jenks	Tulsa	V1	1
R. tibialis	Ardmore	Carter	T2	1
	Magnum	Greer	T2	1
	Stillwater	Payne	T5	1
	Grove	Delaware	T7	1
	Goodwell	Texas	T8	1
	Owasso	Tulsa	T8	1
	Tulsa	Tulsa	T8	1
Coptotermes formosanus	Baton Rouge, LA		outgroup	
Heterotermes aureus	Santa Rita, AZ		outgroup	

der) (GenBank AY380299) were added to the *Reticulitermes* DNA sequences to act as outgroup taxa. The DNA sequences were aligned by the PILEUP program in GCG (Genetics Computer Group, Madison, WI) and adjusted manually. Maximum parsimony analysis on the alignments were conducted with PAUP* 4.0b10 (Swofford 2001). Gaps were treated as missing data. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings and used the Branch and Bound algorithm of PAUP*. Because there are few published accounts of the occurrence of *Reticulitermes* spp. in Oklahoma, we compiled all

available data from existing sources and noted them on our distribution map (Fig. 1).

Results

DNA sequencing of the 16S rDNA amplicon revealed an average size of 428 bp. The average base frequencies were A = 0.41, C = 0.23, G = 0.13, and T = 0.23. The aligned DNA data matrix, including the outgroup taxa, resulted in a total of 433 characters. Of these characters, 86 (20%) were variable and 46 (11%) were phylogenetically informative. From the DNA sequence analysis of *Reticulitermes* spp. samples from 16 Oklahoma counties, a total of 43 *R. flavipes*, four *R. hageni*, one *R. virginicus*, and seven *R. tibialis* were identified based on species diagnostic nucleotide sites (Szalanski et al. 2003) (Table 1, Fig. 1). Morphological identifications yielded the same species identification as the DNA sequences. An additional 10 counties, were included from published anecdotal accounts, bringing the total number of reported counties in Oklahoma to 24 (Fig. 1).

No genetic variation was observed in *R. virginicus*, while two unique haplotypes were found in *R. hageni*, four in *R. tibialis* and 10 in *R. flavipes* (Table 1). Pairwise Tajima-Nei distances (Tajima & Nei 1984) among *Reticulitermes* taxa ranged from 5.7% between *R. flavipes* and *R. hageni*, to 8.3% between *R. flavipes and R. tibialis*. A total of nine nucleotide sites varied among the 10 *R. flavipes* haplotypes (Table 2) and genetic variation among the *R. flavipes* haplotypes ranged from 0.2 to 1.4% (Table 3). The most common haplotypes were L and P with 10 and 14 representatives, respectively. Within *R. tibialis*, a total of three sites varied among the four haplotypes and variation ranged from 0.2 to 0.7%. Within *R. hageni*, one nucleotide site was variable between the two haplotypes.

Bootstrap analysis of the aligned *Reticulitermes* spp. and the outgroup taxa resulted in a consensus tree with several distinct branches (Fig. 2). These distinct clades included *R. flavipes*, *R. hageni* and *R. virginicus*; and *R. tibialis.* Within *R. flavipes*, haplotypes Q and F formed a distinct clade. For *R. tibialis*, haplotypes T8 and T5 formed a distinct clade relative to the two other haplotypes. There was no genetic structure observed among the *R. hageni* haplotypes in the present study.

Discussion

This study updates the geographic distribution of, and genetically classifies, the genus *Reticulitermes* in Oklahoma. However, it does not represent a comprehensive survey of *Reticulitermes* spp. in Oklahoma. Rather, it documents new occurrences of *Reticulitermes* spp. in Oklahoma over a large geographic area. In the present study, genetic divergence values were similar to genetic divergence detected in a study of *Reticulitermes* in Texas (Austin et al. 2004). In terms of population structure, a weak relationship was observed between *R. flavipes* haplotypes Q and F. Haplotype F is distributed throughout the central and eastern portions of the state, while haplotype Q was only observed in Ottawa County, which is located in

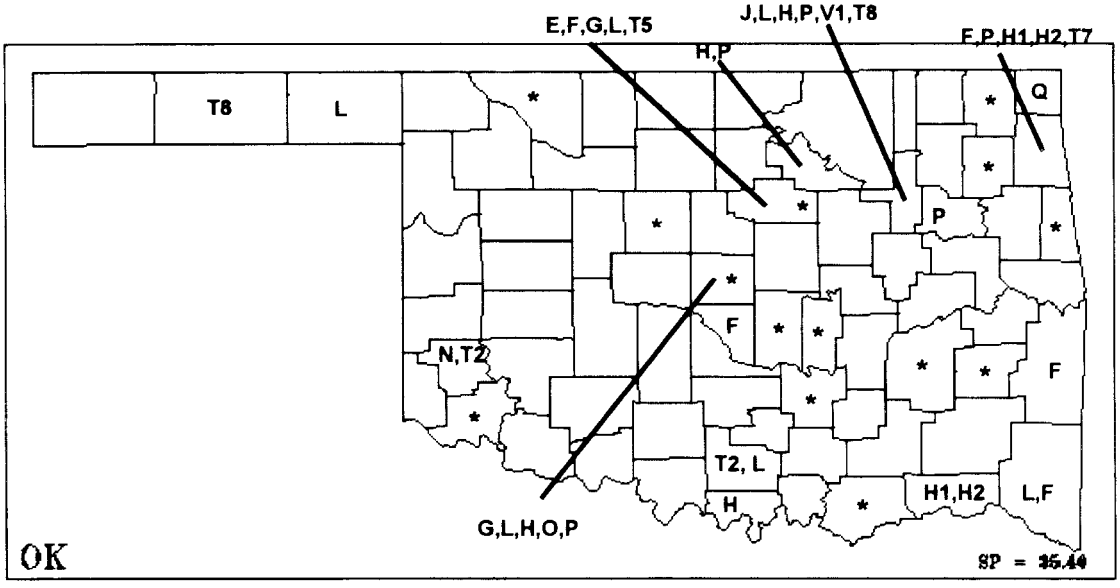

Fig. 1. Distribution of *Reticulitermes* spp. and haplotypes in Oklahoma. Counties designated with an asterisk represent reported cases of *Reticulitermes* spp. but were not used in our genetic analysis and are merely included to update the current distribution of the genus in Oklahoma.

TABLE 2. HAPLOTYPE VARIATION AT NINE NUCLEOTIDE SITES AMONG *RETICULITERMES FLAVIPES* FROM OKLAHOMA.

Haplotype	130	131	158	162	168	179	236	271	274
E	A	A	A	G	A	C	A	T	G
F	*	*	G	*	G	*	C	*	*
G	*	G	G	*	*	*	C	C	*
H	*	*	G	*	*	*	C	*	*
J	*	G	G	*	*	*	C	*	*
L	*	*	G	*	*	G	C	*	*
N	*	*	G	A	*	T	C	*	A
O	T	C	G	A	*	*	C	*	*
P	*	*	G	*	*	*	C	C	*
Q	*	A	G	*	G	T	C	*	G

the northeast corner of Oklahoma. For *R. tibialis* haplotypes T5 and T8 formed a distinct clade, and were collected from two non adjacent counties. In general, there was no population structure for *Reticulitermes* spp. based on genetic haplotypes. Likely reasons for this could be attributed to anthropogenic origins, a lack of nestmate agonism (mixing with non-nestmates imposed by foraging traffic in complex colonies with multiple reproductive centers) (Bulmer & Traniello 2002), or from mixing between different colonies (Clément 1986). In fact, for *R. flavipes*, six of the 10 observed haplotypes (E, F, G, H, J, and L) are shared with Texas. More thorough sampling including termite specimens from more counties are desirable to reveal any existing genetic patterns.

Both *R. virginicus* (Jenks, OK) and *R. hageni* (Grove, OK) were found only in the eastern part of the state where two-thirds of Oklahoma's forest ecosystem consisting of over two million ha of Oklahoma's timberlands (Lewis 2001). This distribution of *R. virginicus* and *R. hageni* in Oklahoma was also observed by Brown et al. (2004). These species are generally found in areas of minimal human disturbance, which may account for their respective occurrences in this study and previous studies. For example, in Arkansas, *R. virginicus* and *R. hageni* are more prevalent in undisturbed habitats (JWA, unpublished). Similarly, the abundance of these species in eastern Oklahoma may indicate central and western Oklahoma represent an east to west transition zone which delimits the westernmost occurrence of *Reticulitermes* species not commonly known from western U.S. states. Interestingly, Oklahoma *R. tibialis* from Ardmore and Stillwater share haplotypes with *R. tibialis* from Texas (T2 and T5, respectively). Also, two of the three *R. hageni* samples are identical to the only southern subterranean termite haplotype observed in Texas (Austin et al. 2004).

Competition between ecologically similar termite species can lead to coexistence through resource partitioning (Houseman et al. 2001). Given *Reticulitermes* ability to hybridize (Clément 1979) and fuse colonies, the opportunity to observe greater genetic diversity is probable, particularly in sympatric zones, where otherwise strong species isolation mechanisms (behavioral, chemical, or temporal) are inadequate to prevent hybridized mating (Austin et al. 2002). Because colony structure and the spatial organization of foraging *Reticulitermes* spp. is less understood, population studies such as this are important in understanding the complex ecology of subterranean termites and *Reticulitermes* spp. in general. By expanding our genetic investigations of *Reticulitermes* spp. from additional geographic zones, the ecological interactions of this genus can be better understood.

TABLE 3. GENETIC DIVERGENCE AMONG *RETICULITERMES FLAVIPES* HAPLOTYPES (HAP) FROM OKLAHOMA.

Hap	Q	F	P	H	J	O	E	G	N
Q	—								
F	0.002	—							
P	0.007	0.005	—						
H	0.005	0.002	0.002	—					
J	0.007	0.005	0.005	0.002	—				
O	0.009	0.007	0.007	0.005	0.005	—			
E	0.009	0.007	0.007	0.005	0.007	0.009	—		
G	0.012	0.009	0.005	0.007	0.005	0.009	0.012	—	
N	0.007	0.009	0.009	0.007	0.009	0.007	0.012	0.014	—

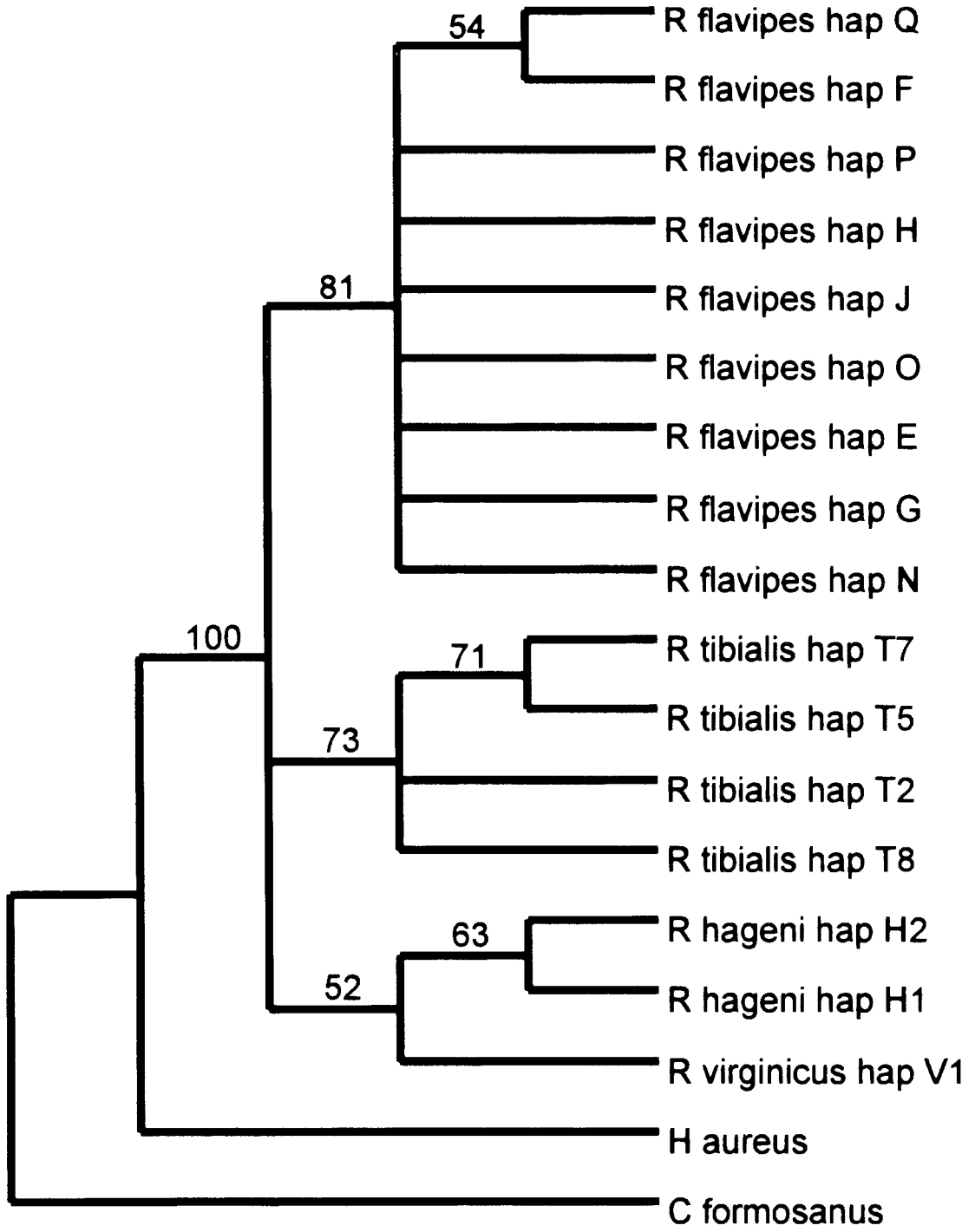

Fig. 2. Single most parsimonious tree during a branch and bound search with PAUP*. Bootstrap values for 1,000 replicates are listed above the branches supported at ≥50%. Tree length = 121, CI = 0.777.

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THE GENUS *GREENIDEA* (RHYNCHOTA: APHIDIDAE) IN THE UNITED STATES

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ABSTRACT

Two species of the Asian genus *Greenidea* have been introduced into the United States, *Greenidea ficicola* Takahashi and *Greenidea psidii* van der Goot. Synonymy confusion between *Greenidea formosana* (Maki) and *G. psidii* is resolved in favor of *G. psidii*. Both species colonize *Ficus* spp., and *G. psidii* colonizes a few other plants, mostly in Myrtaceae. The two species can be distinguished by the ornamentation on the siphunculi on the apterous forms, and usually also by the arrangement of rhinaria on antennal segment III of the alate forms.

Key Words: Rhynchota, Aphididae, *Greenidea ficicola*, *Greenidea psidii*, *Greenidea formosana*, *Ficus*

RESUMEN

Dos especies del género asiático *Greenidea* han sido introducidas a los Estados Unidos, *Greenidea ficicola* Takahashi y *Greenidea psidii* van der Goot. Se resuelve la confusión en la sinónomia entre *Greenidea formosana* (Maki) y *G. psidii* en favor de *G. psidii*. Ambas especies colonizan *Ficus* spp., y *G. psidii* coloniza otras pocas plantas, mayormente las de la familia Myrtaceae. Se puede distinguir las dos especies en la ornamentación de los sifunculi en las formas ápteras, y usualmente también por el arreglo de las rinarias sobre el segmento III de las antenas de las formas aladas.

The Asian genus *Greenidea* (Rhynchota: Aphididae) belongs to the subfamily Greenideinae (Greenideini). Most species of aphids in this subfamily, including those in the genus *Greenidea*, have long siphunculi with correspondingly long setae. Until recently, no species in the subfamily were found in the Western Hemisphere except *Brasilaphis bondari* Mordvilko (Cervaphidini) (Ghosh 1982) native to Brazil, and a fossil species in Dominican amber (Wegierek 2001). Two species, *Greenidea psidii* van der Goot and *Greenidea ficicola* Takahashi, now have been found in the USA. Both species have the potential to become pests of certain ornamental plants.

Greenidea (*Trichosiphum*) *psidii* van der Goot 1916 (= *Greenidea* (*Trichosiphum*) *formosana* (Maki) 1917), **new synonymy** (? = *Greenidea* (*Trichosiphum*) *guangzhouensis* Chang 1979 (Remaudière & Remaudière 1997)) (= *Greenidea* (*Trichosiphum*) *formosana* subsp. *heeri* D. N. Raychaudhuri, M. R. Ghosh, M. Banerjee & A. K. Ghosh 1973 (Remaudière & Remaudière 1997)) (= *Trichosiphum formosanum* Maki 1918)

Greenidea psidii was reported (under *G. formosana*) in Hawaii in 1993 (Beardsley 1993). In 1998, *G. psidii* appeared in California (Gill 1998). Outside of the United States, *G. psidii* is reported from Bangladesh, China, India, Japan, Java, Loochoo Islands (Ryukyus), Nepal, Philippines, Sumatra, and Taiwan (Blackman & Eastop 1994, 2000). I have a specimen from Brisbane, Australia that also appears to be this species.

Reported hosts of *G. psidii* include *Psidium guajava* L. and other Myrtaceae (*Callistemon*, *Eucalyptus*, *Eugenia*, *Melaleuca*, *Metrosideros*, *Rhodomyrtus*, *Syzygium*, and *Tristania*). It also infests *Ficus* (Moraceae), *Engelhardtia* (Juglandaceae), *Scurrula* (Loranthaceae), *Lagerstroemia* (Lythraceae) and *Nesua ferrea* (Clusiaceae) (Beardsley 1993; Blackman & Eastop 1994, 2000; Gill 1998; Noordam 1994).

There is some nomenclatural confusion about *G. psidii*. The most recent catalogue (Remaudière & Remaudière 1997) lists this species as *G. formosana*; however, the most recent revision of the genus (Noordam 1994), lists it as *Greenidea psidii* van der Goot 1917. The Maki description (*G. formosana*) was listed as having been published on October 8, 1917 in honor of the sixtieth birthday of Mr. Yasushi Nawa. In the 1917 journal version of the van der Goot paper (*G. psidii*), it says he finished his work in 1915 and added corrections in January 1916. It seems likely that the book was published early in 1917, but there is no month listed for the publication date, so according to the International Code of Zoological Nomenclature, the date is assumed to be 31 December 1917 (ICZN 2000, Article 21.3.2). However, both the California Academy of Sciences and the library of the Netherlands Entomological Society have copies of an "Extrait," or separate, that lacks a title

page but appears to have been distributed in 1916. In both cases, "1916" has been written on the book. According to the International Code of Zoological Nomenclature, "Before 2000, an author who distributed separates in advance of the specified date of publication of the work in which the material is published thereby advanced the date of publication" (ICZN 2000, Article 21.8). Thus, the species should be *Greenidea psidii* van der Goot 1916.

Greenidea ficicola Takahashi 1921 (= *Greenidea neoficicola* A. K. Ghosh, M. R. Ghosh & D. N. Raychaudhuri (Remaudière & Remaudière 1997))

Greenidea ficicola (Figs. 1 and 2) was first suspected in the Western Hemisphere when a single damaged alate form was collected in a suction trap sample collected 22-27 XI 2002 from Kendall, Florida, near Miami (Florida State Collection of Arthropods (FSCA) #E2002-5901). No other specimens were found until colonies were located on *Ficus aurea* Nutt. on 18 II 2003 (FSCA# E2003-569). There have been several subsequent finds in the Miami area, including more trap collections. The newly established aphid also has been found in Naples, in southwest Florida (FSCA# E2004-810, 849). In addition to Florida, *G. ficicola* is reported from Australia, Bangladesh, Burundi (recent introduction), China, India, Indonesia, Japan, Malaysia, Nepal, Pakistan, Philippines, eastern Russia, and Taiwan (Blackman & Eastop 2000).

Greenidea ficicola seems to be restricted to *Ficus* spp. throughout most of its range; however, in India, there are reports of infestations on *Psid-*

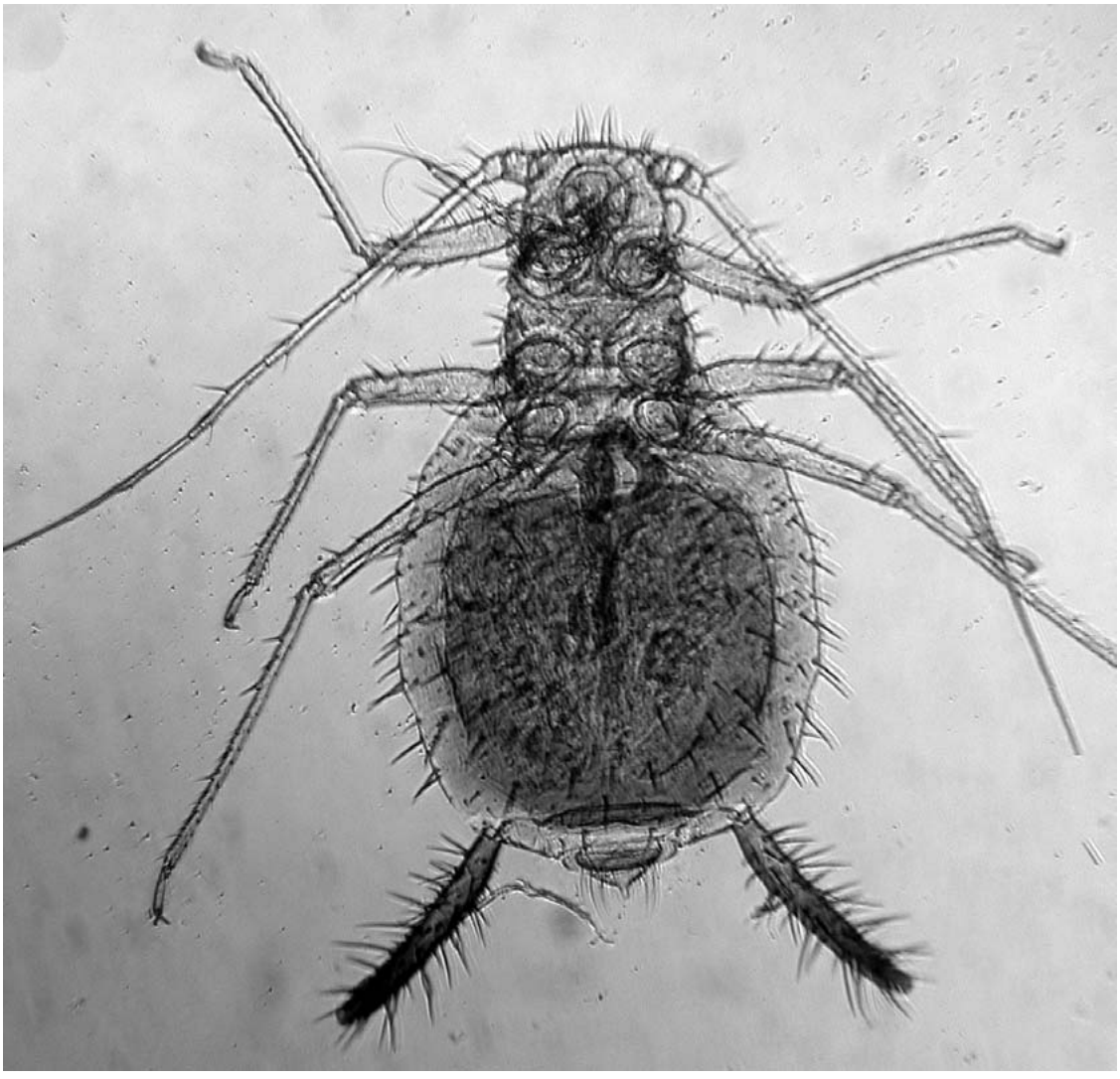

Fig. 1. Adult apterous *Greenidea ficicola* Takahashi.

Fig. 2. Adult alate *Greenidea ficicola* Takahashi.

ium guajava. Noordam (1994) had a collection from *Streblus elongatus* (Miq.) Corner (Moraceae). The record of *G. ficicola* from litchi reported in Blackman & Eastop (2000) is probably spurious (Victor F. Eastop, pers. comm., 12 March 2003). In Florida, we have confirmed field coloni-

zation on *F. aurea* Nutt., *Ficus rigo* (Bailey) Corner, and *Ficus microcarpa* L. f. We were able to rear colonies for several weeks on *Ficus benjamina* L. and *Ficus carica* L. in the laboratory.

Because both species colonize *Ficus* spp., it is important to be able to differentiate between them. A short key is provided:

1a. Apterae with reticulations covering most of the length of the siphunculi (Fig. 3); alatae with 17-21 rhinaria on antennal segment III, in a line and not crowded or touching each other (Fig. 4) . . *G. ficicola*

1b. Apterae with reticulations only at the base of the siphunculi; siphunculi ornamented with irregularly spaced spinules (Fig. 5); alatae with 20-31 rhinaria, some crowded and not in line with the others, often touching (Fig. 6) . . . . . *G. psidii*

Discussion

Both of the newly established species of *Greenidea* have the potential to become pests of certain species of ornamental plants. Both colonize *Ficus*, a genus that includes popular landscape and interiorscape plants, and *G. psidii*

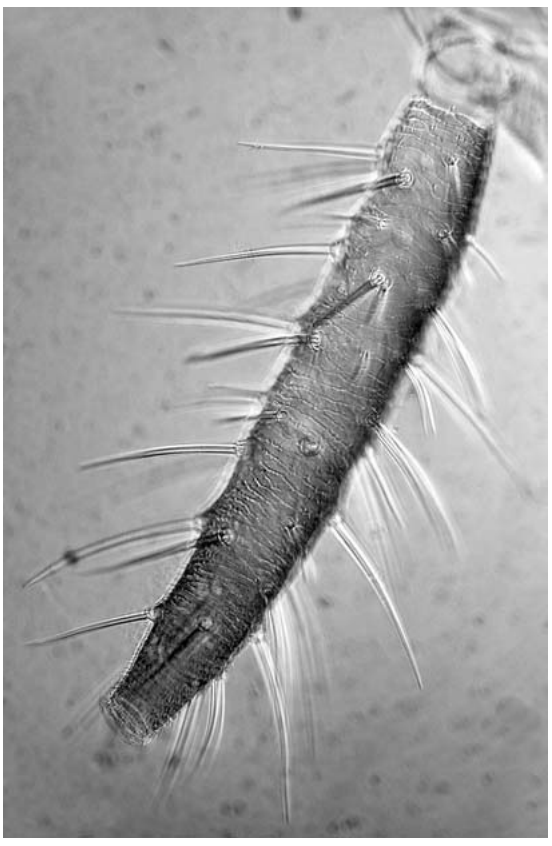

Fig. 3. Siphunculus of apterous *Greenidea ficicola* Takahashi.

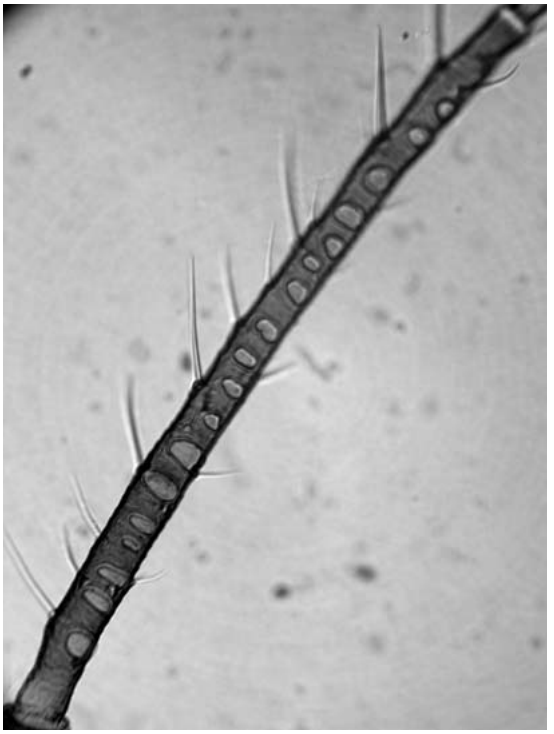

Fig. 4. Antennal segment III of alate *Greenidea ficicola* Takahashi.

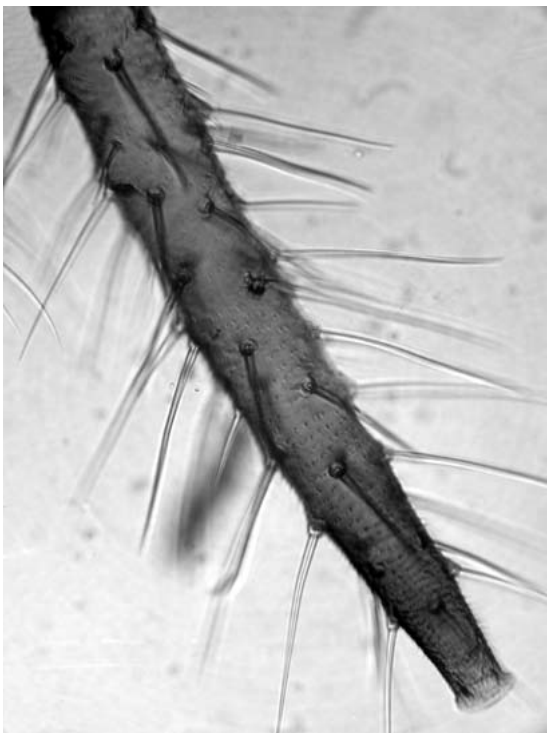

Fig. 5. Siphunculus of apterous *Greenidea psidii* van der Goot (= *G. formosana* (Maki)).

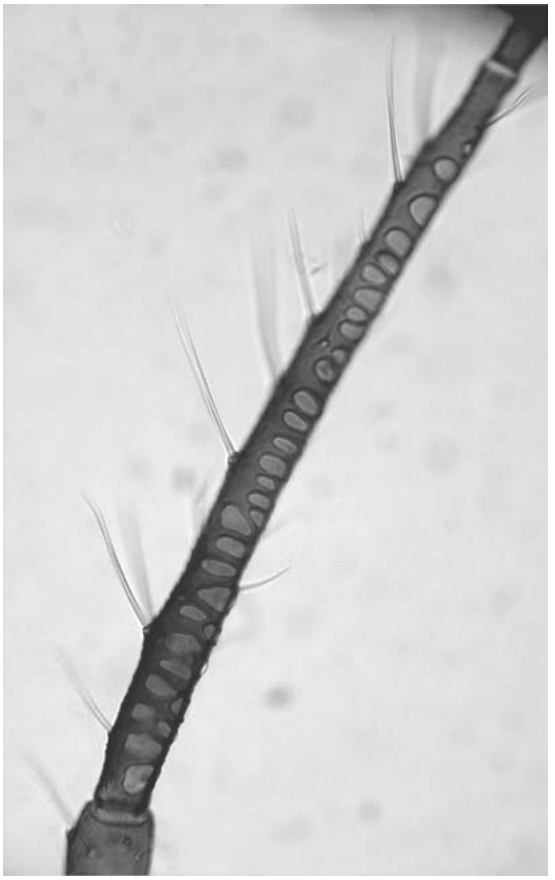

Fig. 6. Antennal segment III of alate *Greenidea psidii* van der Goot (= *G. formosana* (Maki)).

colonizes several species in the Myrtaceae. In the laboratory, *G. ficicola* caused significant leaf drop on *F. benjamina*. *Greenidea psidii* already has been intercepted in Florida in a shipment of *Myrtus communis* L. cut flowers from California (FSCA# E2003-1827). The aphids colonize the buds and new shoots of the host plants. No holocycle is known for either species (Blackman & Eastop 2000), suggesting that freezing temperatures may be limiting, but both species should do well in the neotropics and New World subtropics wherever suitable host plants occur. Interiorscapes, where temperatures do not fall below freezing, also may sustain populations.

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IMMATURE STAGES OF *FOPIUS ARISANUS* (HYMENOPTERA: BRACONIDAE) IN *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

We describe all immature stages, particularly the previously undescribed instars, of *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), an egg-pupal parasitoid of tephritid fruit flies. This is essential for quality control in mass rearing programs and for physiological studies of host-parasite interactions. *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) eggs were parasitized for 24 h and serial collections of hosts were made every 24 h until adults emerged. Immature wasps were dissected from hosts and their mouthhooks and body dimensions measured. Scatter plots of the above measurements and scanning electron microscopy indicated that there are three instars. This contrasts with the four instars previously reported. There appears to be no true fourth instar because the stage immediately following the second instar is indistinguishable from that preceding the prepupal stage.

Key Words: Braconid wasp, tephritid fruit fly host, egg-pupal parasitoid, biological control

RESUMEN

Nosotros describimos todas los estadíos inmaduros, particularmente los estadios no descritos anteriormente, de *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), un parasitoide del huevo-pupal de las moscas de la frutas de la familia Tephritidae. Esto es esencial para el control de cualidad en los programas de cria masiva y para estudios fisiológicos de la interacción entre hospedero y parasitoide. Los huevos de *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) fueron parasitados por 24 horas y hicieron colecciones en serie de los hospederos cada 24 horas hasta que los adultos emergieron. Las avispas inmaduras fueron disectadas de sus hospederos y los ganchos bocales y las dimensiones de cuerpo fueron medidos. Las diagramas de dispersión de las medidas mencionadas y imagenes tomados por el microscopio electrónico (SEM) indicaron que habian tres estadíos. Esto es contrario de los cuatro estadíos reportados anteriormente. Parece que no hay un cuatro estadío verdadero por que el estadío siguiente inmediatamente al segundo estadío es indistinguible del que precede al estadío prepupal.

Fopius arisanus (Sonan) is a parasitoid of many tephritid fruit fly species (Diptera: Tephritidae) including the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Vargas & Ramadan 2000). It is one of the most effective biological control agents of tephritids in Hawaii (Harris & Okamoto 1991) and also parasitizes some New World tephritids such as *Anastrepha suspensa* (Loew) in the laboratory (Lawrence et al. 2000).

Previous reports based on mouthhook dimensions indicated that *F. arisanus* has four instars (Ibrahm et al. 1992). However, there were no illustrations of the larval morphology to facilitate the identification of each instar. Other reports have provided diagrams of the egg, first and fourth instars (Palacio et al. 1992), and the pupa that were useful for their identification, but gave no diagrams of the second or third instars. The goal of this study was to confirm the morphologies of the first and last instars and to describe the previously undescribed intermediate instars of *F. arisanus*.

MATERIALS AND METHODS

Rearing of Parasites

Bactrocera dorsalis eggs were inserted into holes punched into the rind of *Carica papaya* L. and given to adult wasp females aged 10-25 d at a ratio of 20:1 at 75-80°C and 40-50% R.H. under constant light for 24 h. Twenty-four hour sequential collections of hosts were made for 21 d when adult wasps began to emerge. The experiment was duplicated.

Light Microscopy

Fopius arisanus eggs, early instars [1-7 days post parasitism (dpp)], and the heads of late in-

stars (9-14 dpp) were dissected from hosts and placed in fluoromount-G or TE buffer (10 mM Tris, 1 mM EDTA). Other instars (7-9 dpp) were cleared in cellosolve [ethylene glycol monoethyl ether (Carbide and Carbon Chemicals, New York)] for 10 min and mounted with euparal (Barbosa 1974).

Mouthhooks of *F. arisanus* (10 individuals × 2 per time point) and body lengths were measured. The means and standard errors of all measurements were calculated and plotted against one another. The resulting number of aggregations indicated the number of instars according to Dyar's (1890) rule that the "width of the head of a larva in its successive stages follow a regular geometrical progression." Although the rule is applied primarily to lepidopteran larval head capsules, we found that these measurements provided a reliable indicator of instars when used in combination with sequential dissections and other morphological factors.

Scanning Electron Microscopy (SEM)

All larvae were placed in Trump's fixative (1% glutaraldehyde and 4% formaldehyde in phosphate buffer) overnight, washed with 0.1 M cacodylate buffer (3 × 10 min), then fixed in 1% osmium tetroxide for three days. After 3 × 10 min washes in deionized water, the samples were dehydrated in a graded series of ethanol, then incubated in hexamethyldisilazane (HMDS) for 2 × 15 min and air dried (Nation 1983). The larvae were then sputter coated with gold and observed on a Hitachi S-570 scanning electron microscope at 20 kV.

Results

Fopius arisanus eggs measured 300 ±(SE) 11.0 µm (range 250-350 µm) long and 55 ±(SE) 3.0 µm (range 50-75 µm) wide. The egg stage lasted 1-2 d and eggs were observed 0-2 dpp. Scatter plots of mouthhook widths vs. mouthhook lengths (Fig. 1a) and body lengths vs. mouthhook widths (Fig. 1b) show two distinct aggregations of points, the first occurring between 2-8 dpp and the second between 9-14 dpp. The mouthhook, cephalic, and overall morphologies of these two groups correspond to those previously described as first (Fig. 2) and last (fourth) instars, respectively (Ibrahm et al. 1992; Palacio et al. 1992). An instar with overall body size and morphology, differing from the first and last instars, occurred between 7-9 dpp and had no sclerotized mouthhooks (Fig. 3a). This time period coincides with the gap between the two mouthhook size aggregations of the first and last instar and no doubt represents the second instar. Further analysis of the integument of this putative second instar (Fig. 3b) indicated that the integument is distinct from that of the subsequent (last) instar (Figs. 4 and 5).

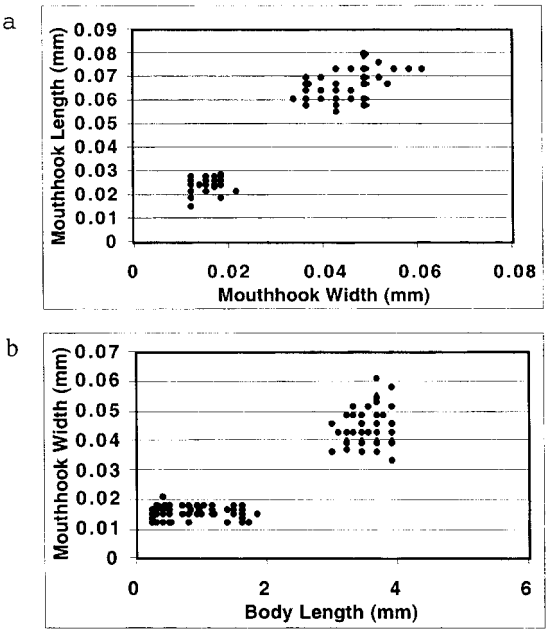

Fig. 1. Scatter plots of (a) mouthhook widths vs mouthhook lengths and (b) body lengths vs mouthhook widths to show distinct aggregations representative of larval instars of *Fopius arisanus* based on Dyar's (1890) rule.

Larval lengths from the tip of the head capsule to the tip of the last abdominal segment were 0.848 ± 0.06 mm (range 0.250-1.84 mm) for first instar, 2.56 ± 0.14 mm (range 1.50-3.22 mm) for second instar, and 3.35 ± 0.10 mm (range 2.99-3.91 mm) for third instar.

The duration of the first, second, and third stadia were eight, two, and six days, respectively. Mouthhook dimensions of the first instar (Fig. 2) were 16 ± 1.0 µm × 24 ± 1.0 µm, second instars had no sclerotized mouthhooks, and third instars had

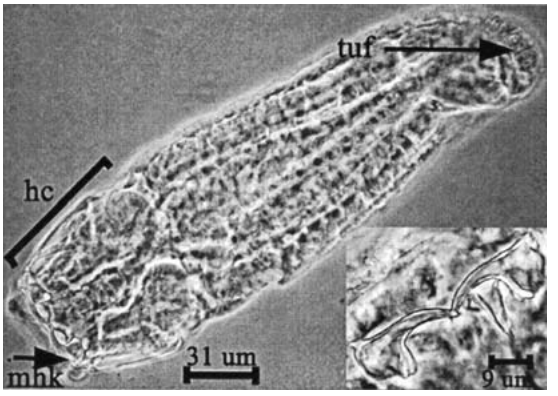

Fig. 2. Light micrographs of first instar (3 dpp) *Fopius arisanus* to show sclerotized head capsule (hc), sclerotized mouthhooks (mhk), and posterior tuft of setae (tuf). Inset = enlargement of mouthhooks.

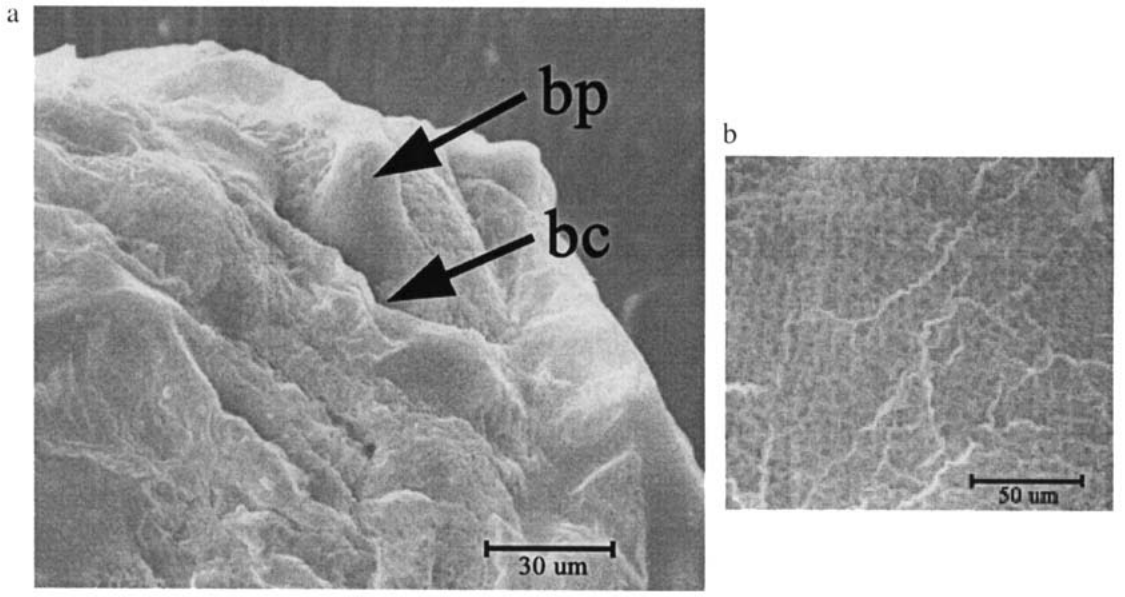

Fig. 3. Scanning electron micrograph of a second instar *Fopius arisanus* (8 dpp) to show (a) cephalic region lacking sclerotized mouthhooks and (b) lack of spines on the integument. bc = buccal cavity; bp = buccal papilla.

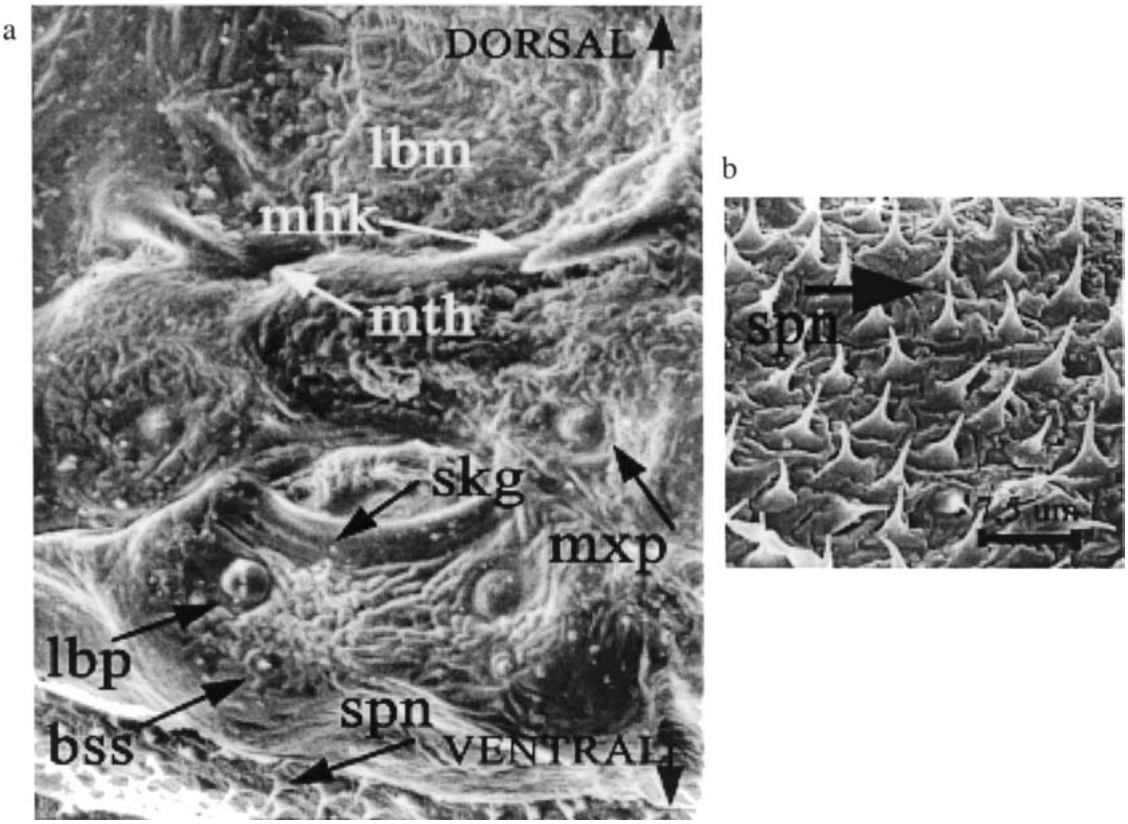

Fig. 4. Scanning electron micrographs of early third instar *Fopius arisanus* (9 dpp) to show cephalic region (a) and spines that cover integument (b). bss = basiconic sensillum; lbm = labrum; lbp = labial palp; mhk = mouthhook; mth = mouth; mxp = maxillary palp; skg = silk gland; spn = spines.

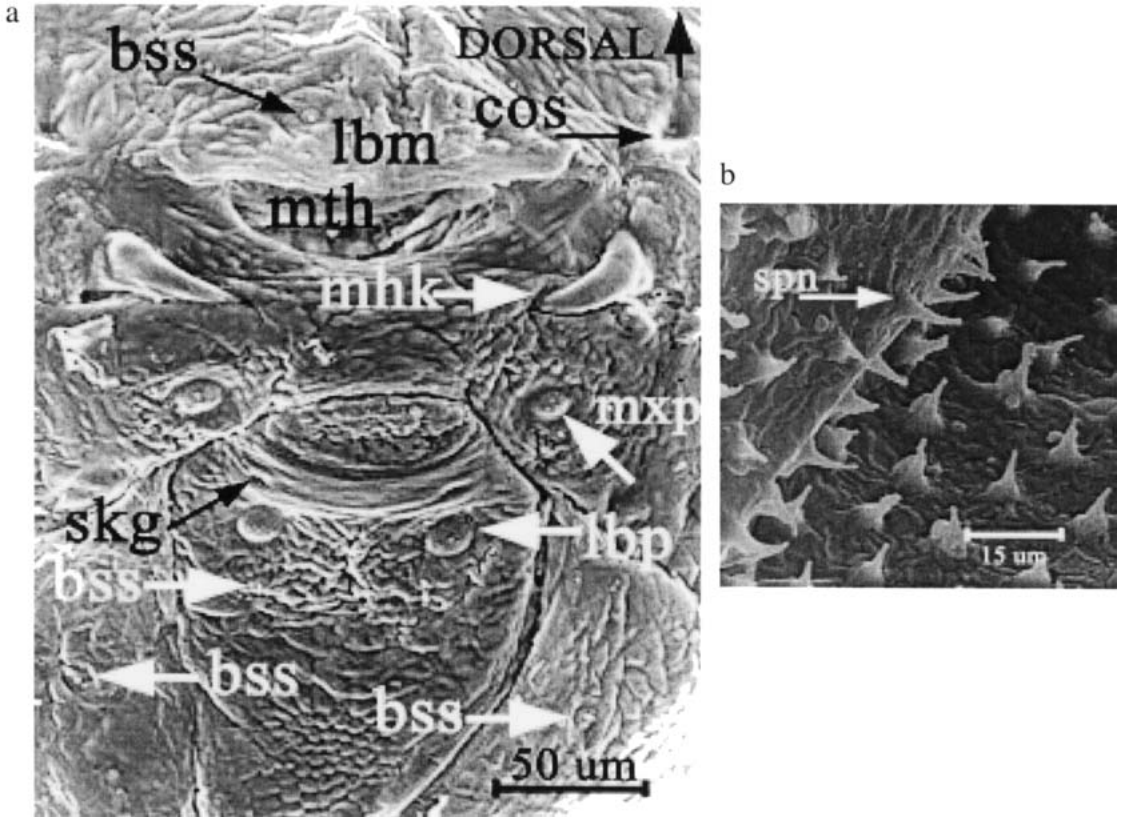

Fig. 5. Scanning electron micrographs of a late third instar (13 dpp) *Fopius arisanus*. (a) cephalic region to show mouthparts. (b) spines that cover integument. bss = basiconic sensillum; cos = coeloconic sensillum; lbm = labrum; lbp = labial palp; mhk = mouthhook; mth = mouth; mxp = maxillary palp; skg = silk gland; spn = spines.

mouthhooks 63 ± 3.0 μm × 42 ± 2.0 μm (3.94× that of first instars.) The distribution of the sensory papillae surrounding the mouthparts of early and late third instars is similar (Figs. 4 and 5).

Discussion

Based on our direct observations of sequentially dissected samples and morphology of *F. arisanus* larvae, we believe that this parasitoid has three instars and a prepupal stage (Fig. 6) because antennal elongation was evident at 13 dpp. While the third instar may have molted to a fourth instar of similar morphology, there was no distinct aggregation of mouthhook dimensions to suggest an increase in size that is normally expected following a larval molt. Although Ibrahm et al. (1992) and Palacio et al. (1992) reported a fourth instar, our SEM evidence indicates that the mouthhooks and cephalic region of the early third instar which occurred immediately after (9-11 dpp) the second instar (7-9 dpp) are similar in morphology and dimension to those of the stage (late third instar) immediately preceding the prepupal stage (13-14 dpp).

Size and duration of parasitoid instars vary with the size, age, and quality of the host in which they are reared (Lawrence et al. 1976; Lawrence 1990). In addition we have observed size differences with different methods of fixation and mounting (P. O. Lawrence, pers. obs.). Consequently, we focused on sclerotized structures such as larval mouthhooks and head capsules because they are reliable characters for identification. Nevertheless, measurements of soft tissues such as body length, in relation to those of sclerotized structures may prove useful for identification. Our larval body measurements vary greatly from those reported by Palacio et al. (1992) and Ibrahm et al. (1992), even though the host species are the same (*B. dorsalis*). This further underscores the unreliability of soft tissue measurements for identifying larval instars of parasitoids in general and *F. arisanus* in particular.

Evaluation of the integuments of the early and late third instars (according to our definition) as well as the pharate pupa, revealed no clear morphological differences. There were no distinctions between mouthhook sizes, integument, antennal and labial sclerites, or distribution of cephalic

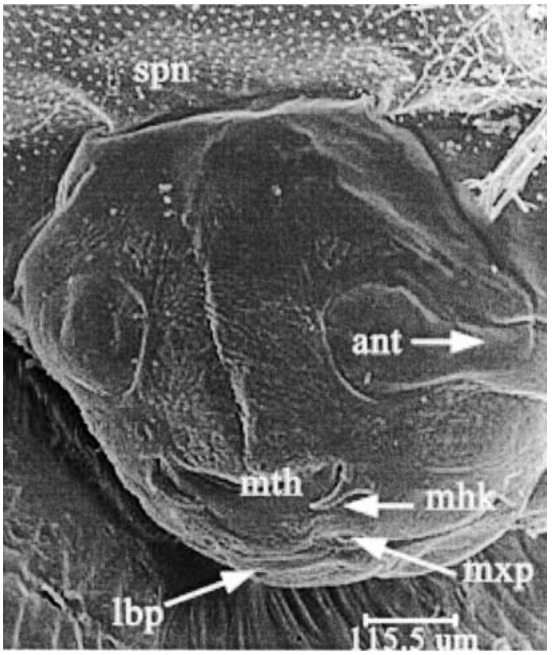

Fig. 6. Scanning electron micrograph of a prepupal *Fopius arisanus* (13 dpp) to show differentiation of antennae (ant). lbp = labial palp; mth = mouth; mhk = mouthhook; mxp = maxillary palp; spn = spines.

sensilla between the early and late third instars. Only direct observation of the molting of second or third instars can definitively distinguish the third from a presumed fourth instar. However, our goal was to establish identification criteria that are useful during dissections for quality control in mass rearing facilities. We believe that standardized sequential sampling along with these morphologies that are also visible under the light microscope will suffice.

Acknowledgments

We thank Donna Williams from the Microbiology and Cell Science Department at the University of Florida for her assistance with SEM and L. Matos and Y. Hashimoto for reviewing the manuscript. Also, financial support from the National Science Foundation (IBN 9986076) to P. O. Lawrence is gratefully acknowledged. This article results from research conducted under CRIS project ENY 03507 and is a Florida Agriculture Journal Series No. R-09948.

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REGIONAL OCCURRENCE OF A SEVERE INFESTATION OF *SIMULIUM SLOSSONAE* (DIPTERA: SIMULIIDAE) ASSOCIATED WITH AN EL NIÑO EVENT IN FLORIDA

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Abstract

A severe infestation of adult host-seeking black flies (Diptera: Simuliidae) occurred in west central Florida during 1998. Collections from stationary suction traps in Pasco County revealed the presence of large numbers of *Simulium slossonae* Dyar and Shannon. This species peaked in traps during March (avg >40 per trap) with a lesser secondary peak in October (avg ≈5 per trap). Moreover, during March, some suction traps had collected as many as 2,000 black flies for the month. It was believed that the spring outbreak of *S. slossonae* was the result of above average precipitation associated with an El Niño event. Precipitation produced by this weather system during the winter of 1997/1998 provided a continuous source of rain-swollen ditches, streams, and creeks for rapid larval and adult production the following spring. Conversely, 1999 resulted in rainfall deficits of 1.5 cm to nearly 7.0 cm below normal. During that year, adult black fly populations were almost nonexistent (≤3 black flies collected per trap month) compared with collections obtained the previous year.

Key Words: Black fly, aquatic arthropods, El Niño, stream ecology

Resumen

Una infestación severa de adultos de la mosca negra (Diptera: Simuliidae) en busqueda de hospederos ocurrió en el oeste central de la Florida durante el 1998. La recolección de moscas en trampas de succión estacionarias en el condado de Pasco reveló la presencia de un alto número de *Simulium slossonae* Dyar y Shannon. El número mas alto de esta especie recolectados en las trampas fue durante el marzo (promedio de >40 por trampa) con el segundo número mas alto en octubre (promedio = 5 por trampa). Además, durante el marzo, algunos de las trampas de succión recollectaron hasta 2,000 moscas negras por el mes. Se cree que la erupción de la población de *S. slossonae* en la primavera fue debido a la precipitación mas alta que el promedio asociada con el evento de El Niño. La precipitación producida por esta sistema de tiempo durante el invierno de 1997/1998 proveyó un fuente continuo de zanjas llenas por la lluvia, quebradas, y arroyos para la producción rapida de las larvas y los adultos en la primavera siguiente. Al contrario, el 1999 resultó en un déficit de lluvia de 1.5 cm hasta casi 7.0 cm menos del normal. Durante aquel año, la población de adultos de la mosca negra fue casi no existente (≤3 moscas negras recolectadas por trampa por mes) comparada con la recolecciones obtenidas el año anterior.

Adult host-seeking black flies (*Simulium* spp.) can often be severe biting pests of humans. The irritation associated with these bites can be considerable and can often make life miserable in areas where black fly populations are in great abundance. Moreover, bites may become itchy and swollen for a number of days. In sensitized individuals reaction to black fly saliva injected at the feeding site may cause a syndrome known as "black fly fever" that consists of headaches, fever, nausea, and/or inflammation of nymph nodes (Harwood & James 1979).

Larval habitats for black flies primarily consist of swift running water, with shallow mountain torrents being favored places (Harwood & James 1979). In Florida, these habitats are not present. Stone & Snoddy (1969) reported that some species prefer slow flowing streams and swamp rivers. These habitats are ubiquitous throughout the State. In 1998, a severe outbreak of adult *Simulium slossonae* Dyar and Shannon occurred in west central Florida (particularly Pasco County). Several reports of chicken mortality caused by adult black fly feeding had been reported in the State during the first three months of that year (Butler & Hogsette 1998). Although this species is primarily a bird feeder, large swarms were often attracted to people causing considerable annoyance (Butler & Hogsette 1998). Because this was an unusual event, the authors

wanted to document seasonal occurrence and abundance of this species in Pasco County. In addition, we discuss the climatic events that led up to that outbreak.

Materials and Methods

Black flies were collected in stationary suction traps, primarily used for mosquito population surveillance, by Pasco County Mosquito Control District (PCMCD) personnel from 1997 through 1999. This trap is similar to that described by Bidlingmayer (1971). Collection data were obtained from daily catches from 35 traps placed throughout the District (covering 855 km^2). In 1998, larval samples from submerged vegetation were periodically obtained from rain-swollen streams by PCMCD staff to determine production sites for emerging adults. Adult and larval samples were sent to Peter Adler, Department of Entomology, Clemson University for identification.

U.S. National Oceanic and Atmospheric Administration monthly total precipitation databases (including monthly normal levels) for 1997-1999 were obtained from their data monitoring station at Tampa International Airport (NOAA 1998b, 1999, 2000).

Results and Discussion

Suction trap collections from 1997, revealed that adult *S. slossonae* were present in Pasco County from May through November at an average of ≤2 black flies per trap month (Fig. 1). In 1998, collections of this species started to increase greatly with a primary peak in March and a slight secondary peak in October. During March, some suction traps had collected nearly 2,000 black flies.

Suction traps located along the Anclote River, Pithlachascotee River, and stream systems in the Starkey Management Area, consistently collected the greatest number of adult *S. slossonae*. These watersheds were probably the primary source of black fly infestation in the County and fit the description by Stone & Snoddy (1969) as slow moving southern swamp rivers/creeks favorable for larval development of this species. Indeed, submerged leaves and branches examined from those watersheds revealed several hundred attached *S. slossonae* larvae.

Simulium slossonae has previously been reported to occur widely in Florida with immature and adult specimens collected throughout the year (Stone & Snoddy 1969; Pinkovsky & Butler 1978; Butler & Hogsette 1998). But black fly populations

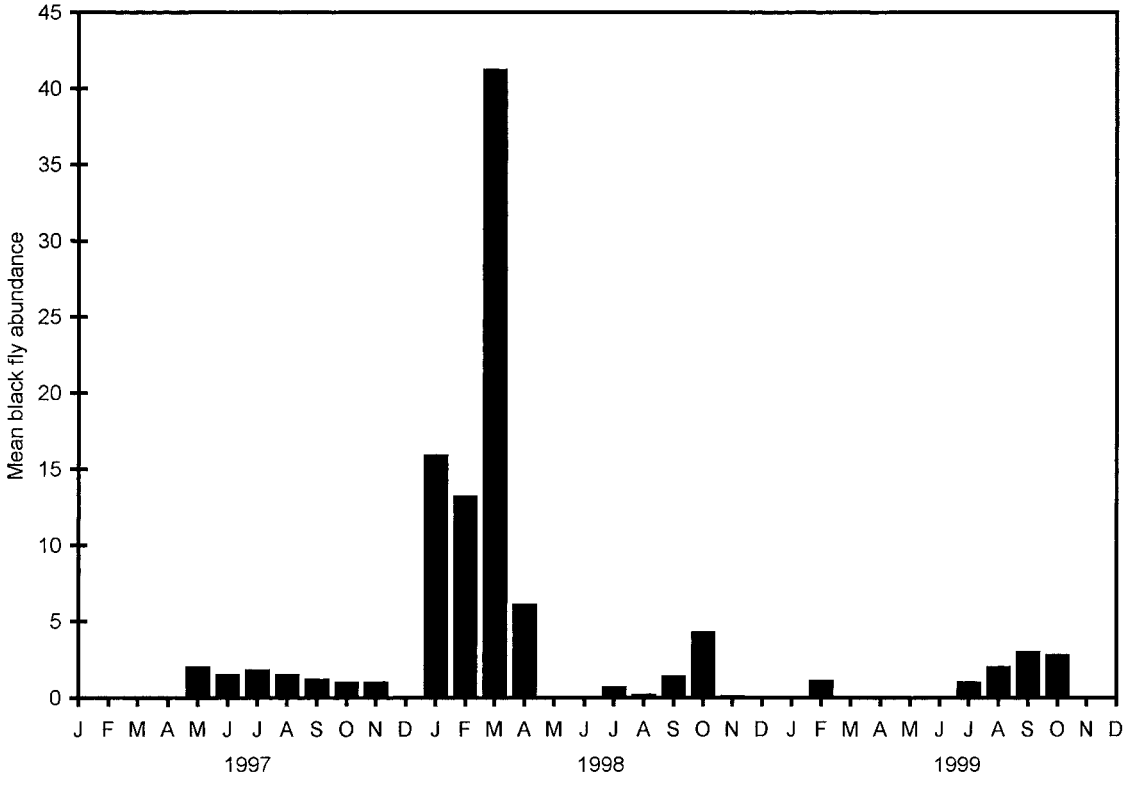

Fig 1. Monthly mean adult *Simulium slossonae* obtained from stationary suction traps, Pasco County, FL, 1997-1999.

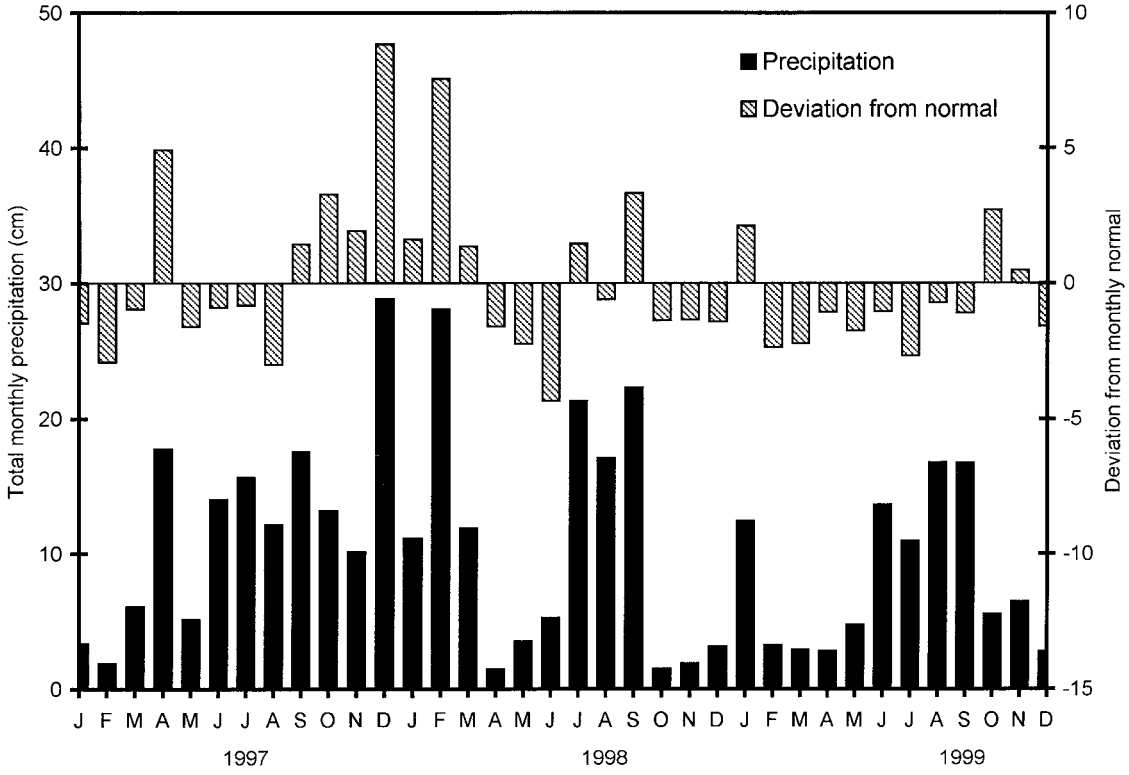

Fig. 2. Monthly total precipitation (cm), and associated deviation from normal, as reported by U. S. National Oceanic and Atmospheric Administration from Tampa International Airport weather data monitoring site, 1997-1999.

reported for the State of Florida had never before increased to the pestiferous levels experienced in 1998. The outbreak of *S. slossonae* during that year appeared to have resulted from above average rainfall during October through December, 1997, and again February, 1998 (Fig. 2). Rainfall was reported to be 8 to 10 times above normal levels in several counties (including Pasco) often swelling stream and river systems to overflow in early 1998 (Morris 1998). Indeed, the National Climatic Data Center (NCDC) reported that the extreme rainfall experienced in central Florida during the latter part of 1997 and beginning of 1998 was associated with El Niño. This event produced 125% to nearly 300% that of normal precipitation levels (NCDC 1998a). According to NCDC, November 1997 to March 1998 had been the wettest reported since records were started in 1895.

During April-June, 1998, adult *S. slossonae* populations had declined considerably (Fig. 1). This period was the driest interval on record (NCDC 1998a). Obviously the precipitation deficits had a limiting effect on larval production (and subsequent adult emergence) through decreased aquatic habitat. During August, rainfall in west central Florida returned to normal or slightly below normal levels (Fig. 2) where, in October, a small peak of adult *S. slossonae* was recorded in traps from Pasco County.

In 1999, adult black fly populations were almost nonexistent (≤3 black flies collected per trap month) (Fig. 1). Larval habitats were not as abundant as the previous year with precipitation levels 1.5 cm to nearly 7.0 cm below normal (Fig. 2).

From our observations, and the data from west central Florida, we found that when above average precipitation events occur in the form of an El Niño weather system, they can trigger a quick build up of adult pestiferous *S. slossonae* populations. Apparently this species can rapidly exploit rain-swollen watershed habitats as larval production areas thereby producing enormous populations of host-seeking adults. Indeed, observations during the first half of 2003, revealed that *S. slossonae* again had risen to pest population levels in Pasco County (J.F.S., unpubl.) by exploiting rain swollen streams produced from another El Niño system during the winter of 2002-2003 (NOAA 2003a, b).

Acknowledgments

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vice, Tallahassee, FL for his help with obtaining central Florida weather-related databases.

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CHOLULA MINUTA, A NEW SPECIES OF MYODOCHINI (LYGAEOIDEA: RHYPAROCHROMIDAE) FROM JAMAICA

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Abstract

A new species of *Cholula* (Myodochini) from Jamaica is described. This represents the first record of this genus for the Caribbean. *Cholula minuta* can be differentiated from other species of the genus mainly by its size. It is one of the smallest species described to date, being similar in size only to *C. parvus*, but *C. minuta* is unicolorous, while *C. parvus* has a mixture of black, brown and white coloration.

Key Words: *Cholula*, Lygaeidae, Rhyparochromidae, Jamaica

Resumen

Se describe una nueva especie de *Cholula* (Myodochini) de Jamaica. Ésta representa el primer registro de este género para el Caribe. *Cholula minuta* puede diferenciarse de otras especies del género principalmente debido a su tamaño. Es una de las especies más pequeñas descritas hasta ahora, es similar en tamaño a *C. parvus*, pero *C. minuta* es de un solo color, mientras que *C. parvus* es de una coloración mezclada de negro, pardo y blanco.

Translation provided by author.

This paper describes a new species of *Cholula* in order to make the name available for a review of West Indian lygaeids that is in preparation by J. A. Slater and R. Baranowski. The genus *Cholula* includes 12 species of Neotropical distribution. None of the species has been recorded previously from the Caribbean; six species are reported from Mexico (*C. bracteicola* Cervantes & Pacheco, *C. irrorandus* (Distant), *C. lactifera* Brailovsky, *C. lympha* Brailovsky, *C. maculatus* (Distant), and *C. scapha* Brailovsky), five from Guatemala (*C. bicolor* Distant, *C. irrorandus*, *C. parvus* (Distant), *C. variegata* Distant, and *C. vigens* (Distant)), three from Panama (*C. discoloria* Distant, *C. firmus* (Distant), and *C. vigens*), and one from Honduras (*C. parvus*) (Brailovsky 1981; Cervantes & Pacheco 2003; Distant 1882-1893).

Cholula minuta Cervantes new species (Fig. 1)

Labium reaching anterior third of abdominal sternite III. Head and anterior pronotal lobe dark ochraceous; posterior pronotal lobe, scutellum, clavus and corium pale ochraceous, with ochraceous punctures. Ventral surface covered with silvery hairs.

Head and anterior pronotal lobe covered with tiny decumbent silvery hairs; eyes and ocelli reddish brown, ocelli located very close to anterior margin of pronotum; antennae pale brown, with joints pale yellow; rostrum pale yellow with tip of segment IV brown. Pronotal collar and lateral margins of posterior pronotal lobe yellow. Pronotum and scutellum very densely punctuate. Acetabulae creamy yellow; coxae ochraceous; femora, tibiae and tarsi pale yellow, fore femur slightly darker. Pro-, meso-, and metapleura pale ochraceous with posterior margins pale yellow. Clavus with three complete rows of punctures and one incomplete row. Corium with two rows of punctures parallel to claval suture; rest of corium with sparse punctures; membrane translucent. Abdominal venter pale ochraceous.

Head slightly declivent, wider than long. Width across eyes greater than width across anterior angles of pronotum. Tylus longer than juga. Lateral pronotal margins sinuate. Disk of scutellum slightly elevated. Fore femur ventrally with double ranked spines. Evaporative area occupying less than half of metapleuron; peritreme auriculate.

Female

Measurements in mm: Body length 3.6; head length 0.57; width across eyes 0.95; interocular distance 0.62; interocellar distance 0.32; postocular distance 0.02; antennal segments: I 0.22, II 0.45, III 0.37, IV 0.7; rostral segments: I 0.46, II 0.52, III 0.3, IV 0.3; pronotal length 0.87, width across humeral angles 1.32, width across anterior margin 0.72; scutellar length 0.68, width 0.68; hind leg: femur length 0.88, tibia length 0.96, tarsi length: I 0.2, II 0.07, III 0.16.

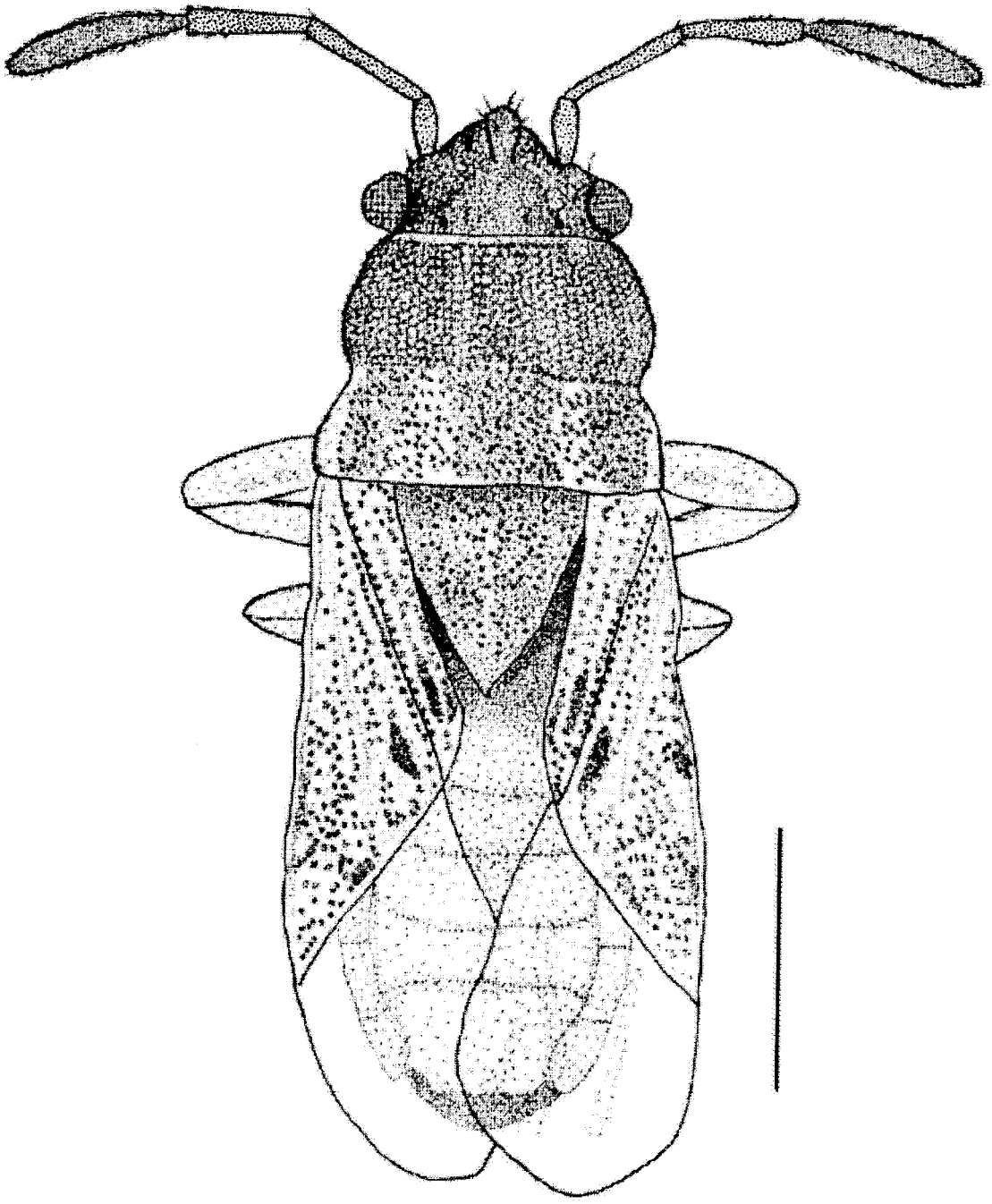

Fig. 1. Adult of *Cholula minuta* new species. The bar at the right indicates the actual size of the male. The female is slightly larger.

Male (Holotype)

Measurements in mm: Body length 3.4; head length 0.5; width across eyes 0.85; interocular distance 0.55; interocellar distance 0.32; postocular distance 0.02; antennal segments: I 0.2, II 0.4, III 0.35, IV 0.65; rostral segments: I 0.45, II 0.48, III 0.35, IV 0.3; pronotal length 0.8, width across humeral angles 1.12, width across anterior margin 0.65; scutellar length 0.68, width 0.68; hind leg:

femur length 0.82, tibia length 0.92, tarsi length: I 0.12, II 0.07, III 0.18.

Types. Holotype, 1♂, JAMAICA: Manchester Parish, Mandeville, 24-VIII-1969, R.E. Woodruff, blacklight trap (Florida State Collection of Arthropods). Paratype. 1♀, same locality as holotype; 23-VIII-1960, J. Howard Frank, blacklight trap (R. Baranowski Collection, University of Florida).

Discussion

This species is similar in coloration to *Cholula lactifera* and *C. bracteicola*, but both species are much larger than *C. minuta* sp. nov. In *C. bracteicola* the rostrum reaches abdominal sternite V, and in *C. minuta* it reaches only to anterior third of abdominal sternite III. In *C. lactifera* antennal segment III is pale ochraceous; in *C. bracteicola* the distal fourth of this segment is dark ochraceous, while in *C. minuta* all antennal segments are pale brown. The hemelytral membrane is transparent in *C. minuta* and in *C. bracteicola*, while in *C. lactifera* it has a milky appearance. *Cholula minuta* is one of the smallest species described to date, being similar in size only to *C. parvus*, but *C. minuta* is unicolorous, while *C. parvus* has a mixture of black, brown, and white coloration.

Recent sampling in Mexico has shown that several species of *Cholula* are arboreal, and are associated with figs, so probably *C. minuta* is also associated with figs in the Caribbean. *Cholula minuta*, as well as other species in the genus, is attracted to light.

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INVERTEBRATE ANIMALS EXTRACTED FROM NATIVE *TILLANDSIA* (BROMELIALES: BROMELIACEAE) IN SARASOTA COUNTY, FLORIDA

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Abstract

Twenty four epiphytic bromeliads belonging to four species (*Tillandsia fasciculata* Swartz, *T. recurvata* (L.), *T. setacea* Swartz, and *T. utriculata* L.) were collected in Sarasota County, Florida, in October-November 1997. Macroscopic invertebrate animals were extracted from each by washing in water, filtering, and preserving in 75% ethanol. Plant sizes were measured in several ways, and their substrate was identified. Invertebrates were sorted, counted, and identified as far as possible to the species level. Two species (*T. fasciculata*, *T. utriculata*) that impound water in their leaf axils housed aquatic dipteran larvae and pupae (Psychodidae, Culicidae, Ceratopogonidae, Chironomidae, Muscidae, and Aulacigastridae) representing 7 species in 6 genera. Only *T. utriculata* had a clear relationship between plant size and number of invertebrates, which was steeper when only aquatic insect larvae were counted. Plants of all four species housed terrestrial invertebrates, representing minimally an additional 82 species in 75 genera and 63 families, very few of which are known to have an obligate relationship with bromeliads, but showing that these plants support a diverse invertebrate fauna. The presence of ant nests in some bromeliads complicated analysis. Such a list of terrestrial invertebrates, identified to the species level, has not before been compiled for bromeliads in Florida. Some collaborating taxonomists obtained specimens of species that they could not identify, including probably undescribed species.

Key Words: Bromeliads, phytotelmata, insects, *Tillandsia utriculata*, bromeliad inhabitants

Resumen

Se colectaron 24 bromeliáceas epífitas que pertenecen a cuatro especies (*Tillandsia fasciculata* Swartz, *T. recurvata* (L.), *T. setacea* Swartz, y *T. utriculata* L.) en el Condado de Sarasota, Florida, durante octubre-noviembre de 1997. Se extrayeron los animales invertebrados macroscópicos de cada planta lavándola en agua y filtrando, seguido por preservación de los especímenes en etanol de 75%. Se midieron los tamaños de las plantas por varios parámetros, y se identificó su sustrato. Los invertebrados se ordenaron, contaron e identificaron tanto posible al nivel de especie. Las dos especies (*T. fasciculata*, *T. utriculata*) que embalsan agua entre sus axilas de hojas alojaron larvas y pupas acuáticas de moscas (Psychodidae, Culicidae, Ceratopogonidae, Chironomidae, Muscidae y Aulacigastridae) representando 7 especies en 6 géneros. Solo *T. utriculata* tuvo una relación clara entre tamaño de planta y cantidad de invertebrados, la cual fue más fuerte cuando se contaron solamente las larvas de insectos acuáticos. Plantas de las cuatro especies alojaron invertebrados terrestres, representando un mínimo de 82 especies adicionales en 75 géneros y 63 familias, muy pocas de las cuales se conocen tener una relación obligada con bromeliáceas, pero demuestran que estas plantas sostienen una diversa fauna de invertebrados. La presencia de nidos de hormigas en algunas bromeliáceas complicó el análisis. Tal lista de invertebrados terrestres, identificados al nivel de especie, no ha sido recopilado anteriormente para bro-

meliáceas en Florida. En este proyecto, varios taxónomos obtuvaron especimenes no-identificados, incluyendo especies probablemente no-descritas.

Translation provided by the authors.

Bromeliads (Bromeliaceae) are a family of at least 2500 species of monocotyledonous plants, almost restricted to the Neotropical region, but including all of Mexico and southernmost USA. The complex architecture of some species traps water in leaf axils (forming phytotelmata) and harbors many species of invertebrate animals. There are thus three types of associations of invertebrates with these plants: (a) those that feed on the plants, (b) organisms aquatic at least in their immature stages, and (c) those terrestrial organisms for which bromeliads provide concealment, humidity, or prey (Frank 1983). Within all three groups are specialists, associated only with bromeliads, as well as generalists that occupy similar (non-bromeliad) habitats.

Four approaches have been followed in attempts to unravel the mysteries of bromeliad fauna. They may be termed (i) brief reports of new discoveries, (ii) in-depth studies (behavioral or ecological or taxonomic) of selected taxa, (iii) whole-fauna inventories, and (iv) broad-scale hypothesis tests. Major difficulties with the last two approaches are the need to involve teams of specialist taxonomists, and of distinguishing transient species from those that have some kind of obligate or at least usual relationship with bromeliads.

In Florida, an inventory of the macroscopic aquatic invertebrate fauna in bromeliad tanks (phytotelmata) is contained in an unpublished Ph.D. dissertation (Fish 1976). A little of the content of that work was reviewed in Frank (1983). An introduction to, and a bibliography of, studies of the fauna and microflora of bromeliad phytotelmata, in Florida and abroad, are WWW-published (Frank 1996 a, b). A complete illustrated key to all developmental stages of all aquatic invertebrates in bromeliad phytotelmata in Florida cannot now be prepared because some species are yet undescribed (unknown to science). In contrast, there are only 16 native species of bromeliads in Florida, identifiable by color photographs online as part of Frank & Thomas (1996) or (for the more botanically adept) by a key in Wunderlin (1998).

In Florida, there has been one inventory of the entire invertebrate fauna in the bromeliad *Tillandsia utriculata* L. (Sidoti 2000), but most of its identifications reached only the level of order. There are works on some insects that feed on and harm bromeliads. Detection of a moth, whose larvae destroy pods of the bromeliad *Tillandsia fasciculata* Swartz, led to a publication about larvae of several moths that occasionally are collected from native bromeliads in Florida (Heppner & Frank 1998). Detection of a Mexican weevil, *Metamasius callizona* (Chevrolat), in Florida led to several publications about bromeliad-eating weevils, reviewed and augmented by Frank (1999) and expanded in two webpages (Larson & Frank 2000; Larson et al. 2001) and two websites (Frank & Thomas 1996; Larson 2000). Notable studies in other countries are by Picado (1913) in Costa Rica, and Beutelspacher (1971a, b) and Palacios-Vargas (1981, 1982) in Mexico.

The Marie Selby Botanical Gardens (MSBG), Sarasota, Florida, have an "Intern Program." Under this program, students interested in plant ecology and other aspects of botany are brought from elsewhere to conduct a short-term (few months) research project in one of these subjects. Margaret Lowman (Research Director, MSBG) and Sheeba Sreenivasan (an intern from Trinidad and Tobago) in 1997 designed a project that was to be a quantitative examination of the invertebrate fauna associated with native bromeliads in Sarasota County. One of us (JHF) was asked to visit MSBG to explain to Sheeba how to extract invertebrates from bromeliads and preserve them for examination, and also to receive her in his laboratory and provide her with literature that would help her to make preliminary identifications of the invertebrate specimens. These were limited to insects, arachnids, myriapods, molluscs, annelids, and the larger crustaceans. Further development depended upon specialist taxonomists to take the preliminary identifications as far as possible to the species level.

In November 1997, Sheeba visited JHF's laboratory at the University of Florida, and used a leaf-area-area meter for about 3 days to measure the leaf-areas of the bromeliads she had collected. To help complete the project, he sorted Sheeba's specimens, some to family, but others only to the level of order. He provided genus- or species-level identification of the immature mosquitoes and a few other dipteran larvae with which he was familiar and, much later, drafted a manuscript for review by the other contributors. All the remaining specimens had to be sent to specialist taxonomists for reliable identification, and the contacts were made and specimens shipped before the end of 1997. Fortunately, taxonomists of the Florida State Collection of Arthropods (FSCA) were receptive to providing help. Here is an account of these invertebrates. This account recognizes the essentiality of the contributions of several taxonomists, who were offered co-authorship (some declined).

Materials and Methods

Twenty four bromeliads were collected from sites in Sarasota County, principally from old-

growth hammocks in the Myakka River State Park, and secondarily two sites in Sarasota. They were removed from various substrates including living trees, dead trees (snags), and a gate (Table 1). While it was being collected, each plant was kept as upright as possible to prevent spillage. The plant was then placed into a polyethylene bag and briefly sprayed with insect repellent before fastening the bag.

Invertebrates were extracted from bromeliads by a variant of the method of Frank et al. (1976). Each plant was cleaned with a jet of water from a hose, with the washings directed into a bucket. The plant was repeatedly submerged and shaken in the bucket before being returned to its bag. The water in the bucket was then filtered with a tea-strainer (mesh size 500 µm) and the residue examined for invertebrates with a dissection microscope. Collected invertebrates were preserved in vials containing 75% ethanol for subsequent identification.

The collected bromeliads included whole specimens of the epiphytic species *Tillandsia utriculata*, *T. fasciculata*, *T. setacea*, and *T. recurvata*. These include all the most widespread of Florida's 16 native species except *T. usneoides* (L.). Only the first two of these impound water in leaf axils, and they do this only when they have reached a certain minimal size (exceeded by the specimens). The volumetric capacity of the water-impounding leaf axils of *T. utriculata* has been related to length of longest leaf and to age (in one habitat) by Frank & Curtis (1981), so length of longest leaf was one of the measurements made (Volumetric capacity in ml = $0.003251 \times$ leaf length in $cm^{2.7799}$). Other measurements were made by dismantling each plant, leaf by leaf, starting from the outermost and working inward. This was done on a white background to facilitate detection of any remaining invertebrates. A component part was considered to be either a leaf or an infructescence (the fruiting phase of an inflorescence). Each element was further designated as live or dead. Each bromeliad's live and dead leaves were counted. All components were refrigerated until leaf and infructescence areas were measured with an area meter (LI-COR Portable Area Meter, model LI 3000, LI-COR Inc., Lincoln, NE, USA). They were then oven-dried for 48 hr before weighing. Table 1, which lists the measurements, is thus a habitat description rather than results.

Results

Table 2 lists the invertebrates collected to the level of family (with number of specimens) for each of the 24 bromeliads sampled. Identification of the invertebrates to the species level, where possible, is given below. Comments are made where deemed appropriate. Three vials containing Mollusca and four with Thysanoptera were mislaid somewhere in the Florida State Collection of Arthropods; details of their contents would not substantially change the conclusions.

Mollusca

None was identified. The three missing vials (6 specimens) may be located in FSCA. No mollusc has a known, obligate relationship to bromeliads. However, H. E. Luther (pers. comm.) has observed snails eating bromeliad trichome caps in the field and greenhouse. Assume minimally one family, one genus, and one species.

Isopoda (identified by G. B. Edwards)

Oniscidae: genus and species unidentified (17 specimens).

Rhyscotidae: genus and species unidentified (9 specimens).

Diplopoda (identified by G. B. Edwards)

Chilognatha: family and genus unidentified, species 1 (12 specimens), species 2 (1 specimen).

Pselaphognatha: Polyxenidae: *Polyxenus fasciculatus* (Say) (5 specimens).

These were collected from all three *Tillandsia* species in MRSP. They have no known relationship to bromeliads. All of the Chilognatha were immature, so could not be identified reliably.

Chilopoda (identified by G. B. Edwards)

Lithobiidae: ?*Neolithobius* sp. (1).

This immature specimen was collected from *T. utriculata* in MRSP. It has no known relationship to bromeliads.

Araneae (identified by G. B. Edwards)

Segestriidae: *Ariadna bicolor* (Hentz) (2).

Theridiidae: *Phoroncidia americana* (Emerton) (1 immature), ?genus (1 immature).

Mysmenidae: *Mysmenopsis cymbia* Levi (10).

Linyphiidae: *Ceraticelus* ?*phylax* Ivie & Barrows (1 female).

Tetragnathidae: *Dolichognatha pentagona* (Hentz) (1), ?*D. pentagona* (1 immature, damaged), ?*Tetragnatha* sp. (2 immatures).

Lycosidae: ?genus (2 immatures).

Pisauridae: ?*Dolomedes* sp. (1 immature).

Agelenidae: ?*Agelenopsis* sp. (1 immature).

Hahniidae: *Hahnia okefinokensis* Chamberlin & Ivie (1).

Dictynidae: *Emblyna capens* Chamberlin (1), *Emblyna* sp. (2 immature), *Lathys delicatula* Gertsch & Mulaik (2).

Anyphaenidae: *Lupettiana mordax* (O.P. Cambridge) (1).

Liocranidae: *Scotinella pintura* (Ivie & Barrows) (3), *Scotinella* sp. (1 immature).

TABLE 1. BROMELIAD MEASUREMENTS (NUMBER OF LIVE LEAVES, DEAD LEAVES, LONGEST LEAF, AREAS OF LIVING AND DEAD LEAVES, OF LIVING AND DEAD INFRUCTESCENCES, AND DRY WEIGHTS) MATCHED TO SAMPLE NUMBER (CODE), SUBSTRATE ON WHICH IT GREW, AND IDENTIFICATION.

Code	Substrate	No. live leaves	No. dead leaves	Longest leaf (cm)	Live leaf area (cm^2)	Dead leaf area (cm^2)	Live infr. area (cm^2)	Dead infr. area (cm^2)	Dry wt (g)
				Tillandsia fasciculata					
8	*Cephalanthus*	64	4	58.4	2621.99	60.18	0	0	57.5
9	*Cephalanthus*	65	20	62.0	2794.81	316.20	0	0	71.5
10	*Cephalanthus*	51	11	46.4	898.86	66.24	0	0	21.5
15	snag	55	13	44.5	2002.08	199.07	0	0	33.0
17	*Ulmus*	33	3	44.5	881.81	44.55	0	0	10.5
18	fallen branch	55	2	22.8	350.46	37.23	0	0	6.5
19	rooted in soil	58	10	29.2	475.15	27.79	0	0	11.5
20	*Quercus*	76	13	55.4	5006.87	371.90	0	0	189.5
				Tillandsia utriculata					
1	snag	41	6	32.0	531.62	38.44	0	0	8.0
2	snag	56	29	46.5	1793.91	237.25	0	0	32.0
3	snag	55	22	33.0	824.92	63.65	0	0	11.5
4	snag	62	11	17.9	164.69	14.34	0	0	2.5
16	*Quercus*	60	12	91.1	9285.81	909.66	0	0	147.0
24	*Quercus*	59	11	93.3	7350.48	0	0	0	152.5
				Tillandsia recurvata					
21	*Sabal*	1140	71	10.1	672.1	48.60	0	0	10.5
22	wooden gate	80	1	9.3	51.69	2.37	6.02	0	1.0
23	*Quercus*	537	58	13.5	305.72	82.25	34.36	23.00	5.0
				Tillandsia setacea					
5	snag	196	82	19.0	151.37	62.86	0	0	3.5
6	snag	349	24	18.6	236.57	17.15	0	0	3.0
7	snag	158	22	27.2	210.06	27.90	28.20	5.58	8.0
11	*Cephalanthus*	419	63	32.1	556.39	60.18	67.68	7.97	16.5
12	*Cephalanthus*	338	29	28.9	437.23	62.32	18.87	16.13	9.5
13	*Cephalanthus*	81	34	23.5	185.37	36.46	26.92	0	3.0
14	*Quercus*	379	32	29.8	298.02	18.81	4.93	5.33	8.5

Clubionidae: *Clubiona pygmaea* Banks (3), *Clubiona* sp. (1 immature), *Elaver excepta* (L. Koch) (10).

Gnaphosidae: *Litopyllus cubanus* Bryant (1), *Sergiolus* sp. (1 immature).

Sparassidae: *Pseudosparianthis cubana* Banks (1 immature).

Thomisidae: *Bassaniana floridana* (Banks) (1), *Bassaniana* sp. (4 immatures).

Salticidae: *Anasaitis canosa* (Walckenauer) (2).

The spiders seem to represent 21 species, in 21 genera and 17 families. For only one spider (*Pelegrina tillandsia* [Kaston]) is a bromeliad (*Tillandsia usneoides*) in the southern USA known to be the preferred habitat. In the Neotropical region, however, other spiders typically inhabit bromeliads and even are semi-aquatic in bromeliad leaf axils.

Pseudoscorpiones (identified by G. B. Edwards)

Chthoniidae: genus and species unidentified (2 immatures).

One specimen was from *T. fasciculata* and the other from *T. setacea*. They were unidentifiable because immature. Pseudoscorpions have no known obligate relationship to bromeliads.

Acari (identified by W. C. Welbourn)

Liodidae: *Liodes* sp. 1 (16), *Liodes* sp. 2 (13), *Liodes* sp. 3 (3).

Ascidae: *Lasioseius* sp. (2).

Haplozetidae: genus and species unidentified (1).

Oripodidae: genus and species unidentified (1).

Uropodidae: *Uropoda* sp. (2).

Oppiidae: genus and species unidentified (1).

Orbataloid: genus and species unidentified (1).

Histiostomatidae: *Hormosianoetus* sp. (37).

None of these 10 species in 8 genera and 8 families is known to have any obligate relationship with bromeliads. There is a pressing need for more basic taxonomic work on Floridian Acari other than those of economic importance; only then will specimens be identifiable to the species level.

TABLE 2. ORIGIN (CODE/PLACE) OF *TILLANDSIA* SPECIMENS AND NUMBERS OF ARTHROPODS EXTRACTED, TO FAMILY LEVEL. ALL WERE COLLECTED IN MID-OCTOBER TO MID-NOVEMBER 1997 IN SARASOTA COUNTY, FL.

CD/PL	Fauna to level of family, and number of specimens	Sum
	Tillandsia fasciculata	
08/ M	CRUSTACEA: Isopoda: Rhyscotidae (2), ARACHNIDA: Araneae: Theridiidae (1), Mysmenidae (10), Hahniidae (1), Liocranidae (1), INSECTA: Homoptera: Aphididae (1), Lepidoptera: Tineidae (larvae, 1), Diptera: ?Muscidae (larva 1), Hymenoptera: Formicidae (1)	19
09/ M	ARACHNIDA: Araneae: Tetragnathidae (1), Dictynidae (1), Liocranidae (1), Clubionidae (2), Salticidae (1), INSECTA: Isoptera: Kalotermitidae (4), Blattodea: Blatellidae (2), Psocoptera: Lepidopsocidae (nymphs 2), family indet. (nymphs 2), Diptera: ?Muscidae (larva 1), Hymenoptera: Formicidae (1)	18
10/ M	CRUSTACEA: Isopoda: Rhyscotidae (6), ARACHNIDA: Araneae: Linyphiidae (1), Agelenidae (1), Clubionidae (2), Acari: Uropodidae (1), INSECTA: Orthoptera: Gryllidae (3), Psocoptera: Lepidopsocidae (1), Archipsocidae (1), Peripsocidae (2), family indet. (1), Coleoptera: Tenebrionidae (1), Hymenoptera: Formicidae (1)	21
15/ M	DIPLOPODA: Pselaphognatha: Polyxenidae (1), ARACHNIDA: Araneae: Segestriidae (1), Pisauridae (1), Acari: Histiostomatidae (19), INSECTA: Thysanoptera (1), Psocoptera: Lepidopsocidae (1), Orthoptera: Gryllidae (2), Coleoptera (larvae 3, of 3 families), Diptera: Psychodidae (larvae 32), Culicidae (larva 1), Ceratopogonidae (larvae 3)	65
17/ M	CRUSTACEA: Isopoda: Rhyscotidae (1), DIPLOPODA: Chilognatha: family indet. (1), ARACHNIDA: Araneae: Clubionidae (2), Pseudoscorpionida: Chthoniidae (1), INSECTA: Blattodea: Blatellidae (4, and one egg case), Lepidoptera: family indet. (larvae 5), Coleoptera: Carabidae (larva 1), Diptera: Ceratopogonidae (larva 1)	17
18/ M	ARACHNIDA: Araneae: Anyphaenidae (1), Thomisidae (1), Salticidae (1), Acari (?1), INSECTA: Orthoptera: Gryllidae (1), Coleoptera: Scirtidae (1), Hymenoptera: Formicidae (1)	7
19/ M	CRUSTACEA: Isopoda: Oniscidae (3), DIPLOPODA: Pselaphognatha: Polyxenidae (3), Pselaphognatha: family indet. (3), ARACHNIDA: Araneae: Lycosidae (2), INSECTA: Blattodea (egg case 1), Homoptera: Ortheziidae (1), Coleoptera: Brentidae (1), Hymenoptera: Formicidae (128 plus brood), Ichneumonidae (2)	144
20/ M	MOLLUSCA (3), CRUSTACEA: Isopoda: Oniscidae (10), ARACHNIDA: Araneae: Dictynidae (1), Sparassidae (1), INSECTA: Blattodea: Blattidae (1), Orthoptera: Gryllidae (1), Lepidoptera: family indet. (larva 1), Diptera: Psychodidae (larvae 2), Ceratopogonidae (larvae 8, pupae 2) Chironomidae (larvae 4), Aulacigastridae: (larva 1, pupa 1)	36
	Tillandsia utriculata	
01/ M	ARACHNIDA: Araneae: Dictynidae (1), Acari: Liodidae (1)	2
02/ M	CRUSTACEA: Isopoda: Oniscidae (2), DIPLOPODA: Chilognatha: family indet. (5), ARACHNIDA: Araneae: Tetragnathidae (1), Liocranidae (2), Clubionidae (1), Acari: Ascidae (2), INSECTA: Blattodea: Blatellidae (1), Psocoptera: Pseudocaeciliidae (1), Liposcelidae (1), family indet. (nymph 1), Diptera: Ceratopogonidae (larva 1), Psychodidae (larvae 16), Hymenoptera: Formicidae (5)	39
03/ M	DIPLOPODA: Pselaphognatha: Polyxenidae (1), ARACHNIDA: Araneae: Clubionidae (1), Thomisidae (1), INSECTA: Psocoptera: Caeciliusidae (1), Archipsocidae (1), Liposcelidae (1), Lepidopsocidae (nymph 1), family indet. (nymph 1), Blattodea: Blattidae (2), Homoptera: Coccidae (1), Coleoptera: Curculionidae (3), Diptera, Ceratopogonidae (larvae 2)	16
04/ M	DIPLOPODA: Chilognatha: family indet. (2), ARACHNIDA: Araneae: Clubionidae (1), Thomisidae (2), Acari: Liodidae (1), INSECTA: Blattodea: Blatellidae (2), Coleoptera: Curculionidae (1), Diptera: Chironomidae (1)	10
16/ M	CHILOPODA: Lithobiidae (1), DIPLOPODA: Chilognatha: family indet. (1), ARACHNIDA: Acari: Histiostomatidae (18), INSECTA: Blattodea: Blattidae (1), Psocoptera: family indet. (1 nymph), Thysanoptera (2), Lepidoptera: family indet. (larva 1), Diptera: Psychodidae: (larvae 108, pupa 1), Culicidae: (larvae 28), Ceratopogonidae (larvae 47), Chironomidae (larvae 3), Aulacigastridae: (5), Cecidomyiidae (adults 2, pupa 1), Hymenoptera: Formicidae (6)	226
24/ S	ARACHNIDA: Araneae: Dictynidae (1), Gnaphosidae (1), INSECTA: Collembola: Entomobryidae (22), Psocoptera: Trogiidae (2), family indet. (nymph 1), Coleoptera: Coccinellidae (1), Diptera: Psychodidae (larvae 31), Hymenoptera: Formicidae (76, of 2 spp., each with brood)	135
	Tillandsia recurvata	
21/ S	INSECTA: Psocoptera: Lepidopsocidae (1), Coleoptera: Elateridae (1), Hymenoptera: Formicidae (2)	4
22/ S	No animals were collected	0

TABLE 2. (CONTINUED) ORIGIN (CODE/PLACE) OF *TILLANDSIA* SPECIMENS AND NUMBERS OF ARTHROPODS EXTRACTED, TO FAMILY LEVEL. ALL WERE COLLECTED IN MID-OCTOBER TO MID-NOVEMBER 1997 IN SARASOTA COUNTY, FL.

23/ S	MOLLUSCA (1), ARACHNIDA: Araneae: Gnaphosidae (1), INSECTA: Hemiptera: Miridae (nymphs 2), Psocoptera: Trogiidae (1), Hymenoptera: Formicidae (1)	6
	Tillandsia setacea	
05/ M	Diplopoda: Pselaphognatha: Polyxenidae (1), ARACHNIDA: Acari: Liodidae (4), Haplozetidae (1), Oripodidae (1), INSECTA: Psocoptera: Peripsocidae (1), Archipsocidae (1 nymph), Lepidopsocidae (1 nymph), Orthoptera: Gryllidae (1)	11
06/ M	ARACHNIDA: Araneae: Clubionidae (2), Thomisidae (1), Acari: Liodidae (9), INSECTA: Psocoptera: Peripsocidae (2), Homoptera: Aphididae (4), Hymenoptera: Aphelinidae (1)	19
07/ M	MOLLUSCA (2), ARACHNIDA: Araneae: Segestriidae (2), INSECTA: Blattodea: Blatellidae (2), Lepidoptera: family indet. (larvae 2), Hymenoptera: Formicidae (52)	60
11/ M	ARACHNIDA: Araneae: Dictynidae (1), Clubionidae (1), Acari: Liodidae (2), INSECTA: Thysanoptera (1), Coleoptera: Scirtidae (1)	6
12/ M	ARACHNIDA: Araneae: Theridiidae (1), Tetragnathidae (2), Thomisidae (1), Acari: Liodidae (2), Oppiidae (1), 'orbataloid' (1), Pseudoscorpionida: Chthoniidae (1), INSECTA: Collembola: Hypogastruridae (5), Thysanoptera (2), Psocoptera: Peripsocidae (nymph 1), Hymenoptera: Formicidae (1)	18
13/ M	CRUSTACEA: Isopoda: Oniscidae (2), ARACHNIDA: Araneae: Clubionidae (1), Acari: Liodidae (13), INSECTA: Psocoptera: Liposcelidae (1), Diptera: Cecidomyiidae (1)	18
14/ M	ARACHNIDA: Araneae: Clubionidae (1), Acari: Uropodidae (1), INSECTA: Lepidoptera: Gelechiidae (larva in flower) (1), Hymenoptera: Formicidae (1)	4

Notes: CD = code number (collection no.)/PL = place (M = Myakka River State Park, S = Sarasota). SUM = total number of invertebrate animals of the groups sampled. Presence of immature stages suggests that development was taking place in the bromeliad unless the individuals fell from the tree above.

Collembola (identified by R. J. Snider)

Entomobryidae: *Seira steinmetzi* Wray (22).

Hypogastruridae: *Xenylla* sp. (5).

The specimens of *Xenylla* represent an undescribed species and were retained by R. J. Snider.

Orthoptera (identified by T. J. Walker)

Gryllidae: *Cycloptilum trigonipalpum* (Rehn & Hebard).

All 8 specimens of Orthoptera were identified as of this species or were unidentifiable because immature, but probably belong to this species. Seven of them were collected from *T. fasciculata* and one from *T. setacea*, all within MRSP.

Blattodea (identified by M. C. Thomas)

Blattellidae: *Cariblatta* sp. prob. *lutea* (Saussure & Zehnter) (12).

Blattidae: *Eurycotis floridana* (Walker) (4).

Neither species has any known obligate relationship with bromeliads.

Isoptera (identified by R. H. Scheffrahn)

Kalotermitidae: *Crytotermes ?cavifrons* Banks (4).

No termite is known to have an obligate relationship with bromeliads. Most likely these had fallen from a dead tree limb.

Psocoptera (identified by E. L. Mockford)

Trogiidae: *Cerobasis guestfalica* (Kolbe) (4).

Lepidopsocidae: *Echmepteryx* (*Thylacopsis*) *madagascariensis* (Kolbe) (3), *Echmepteryx* sp. (1), *Nepticulomima* sp. (1), unidentified nymphs (4).

Liposcelidae: *Liposcelis ornata* Mockford (1), *Liposcelis* sp. (2).

Archipsocidae: *Archipsocus* sp. (2), unidentified nymphs (1).

Peripsocidae: *Peripsocus* sp. (5).

Caeciliusidae: *Valenzuela indicator* (Mockford) (= *Caecilius indicator* Mockford) (1).

Pseudocaeciliidae: *Pseudocaecilius citricola* (Ashmead) (1).

Epipsocidae: *Mesepipsocus niger* (New) (3), unidentified nymphs (4).

None of these 12 species in 9 genera and 8 families is known to have an obligate relationship to bromeliads. As in so many other groups, it is difficult to identify nymphs reliably to the species level. The surprise among these specimens was the finding of specimens of *C. guestfalica* from two *Tillandsia* specimens (*T. recurvata*, *T. utriculata*) from the city of Sarasota; it is an adventive species.

Thysanoptera

None was identified. The four missing vials (6 specimens) may be located in FSCA. Assume minimally one family, one genus, and one species.

Hemiptera/Homoptera (identified by S. E. Halbert) (Ortheziidae by A. B. Hamon)

Miridae: unidentified nymphs.

Aphididae: *Myzocallis* sp. (4), unidentified genus (1 cast skin).

Ortheziidae: *Orthezia* sp. (1 nymph).

Coccidae: genus and species unidentified (1 adult male).

The *Myzocallis* aphids are known to feed on oak; one of the specimens was parasitized by *Aphelinus* sp. (Hymenoptera: Aphelinidae) (det. G. A. Evans, FSCA). The only species of *Orthezia* reported from *Tillandsia* in Florida is *O. tillandsiae* Morrison, but the specimen obtained (from *T. fasciculata* in MRSP) is immature and could not be identified with certainty. The Miridae were unidentifiable because they were immature.

Coleoptera (identified by M. C. Thomas)

Scirtidae: *Ora* sp. (1), *Cyphon* sp. (1).

Elateridae: *Conoderus amplicollis* (Gyllenhal) (1).

Tenebrionidae: *Glyptotus cribratus* LeConte (1).

Brentidae: *Apion* sp. (1).

Curculionidae: *Acalles clavatus* Say (3); *Conotrachelus maritimus* Blatchley (2).

Larvae of Scirtidae are aquatic. It is possible that the two adults of Scirtidae are associated with bromeliad phytotelmata, but both specimens were found in *T. setacea*, which does not form phytotelmata. Alternatively, their larvae may develop in treeholes. Specimens mislaid include one adult of Coccinellidae and several beetle larvae, of which one is of Carabidae.

Lepidoptera (identified by D. H. Habeck)

Tineidae: genus and species unidentified (1 larva).

Gelechiidae: genus and species unidentified (1 larva).

Family uncertain: (8 larvae).

Lepidoptera were represented only by larvae of "primitive" families. Their identification was uncertain. It is not clear whether these larvae were feeding on bromeliads, on debris in the bromeliads, or on the tree canopy (or epiphytes) above. The gelechiid larva was found clinging to a flower (which was not a bromeliad flower) in the bromeliad, but there is no evidence it was feeding on that flower. There is a clear need to rear lepidopteran larvae encountered in bromeliads, to allow identification of adults and associate larvae with adults.

Diptera (identified by J. H. Frank) (Cecidomyiidae by R. J. Gagné, Ceratopogonidae by G. J. Steck)

Cecidomyiidae: *Campylomyza* sp. (1 adult), *Lestodiplosis laticaulis* Gagné (2 adults and 1 pupa).

Psychodidae: *Alepia* sp. (190 larvae and pupae).

Culicidae: *Wyeomyia mitchellii* (Theobald) (25 larvae), *W. vanduzeei* Dyar & Knab (3 larvae).

Ceratopogonidae: *Forcipomyia* sp. or spp. (62 larvae, 2 pupae)

Chironomidae: *Monopelopia tillandsia* Beck & Beck (53 larvae), (1 damaged unidentified adult).

Aulacigastridae: *Stenomicra* (7 larvae).

?Muscidae: genus and species unidentified (2 larvae) (perhaps this is the *Neodexiopsis* sp. of Fish [1976]).

Specimens of Cecidomyiidae were shipped in vials of alcohol to R. A. Gagné; he found both species interesting and retained one specimen of each. He reports that *L. laticaulis* is known as a predator of *Diaspis echinocacti* (Bouché) (Homoptera: Diaspididae) a scale insect on *Opuntia* cacti—so its presence in *T. utriculata* is unexpected. Larvae of Psychodidae, Culicidae, many Ceratopogonidae, Chironomidae, and Aulacigastridae are aquatic and, expectedly, were found only in *T. fasciculata* and *T. utriculata*. Identification of these larvae was made by J. H. Frank (who makes no claim to be an expert on larvae of Diptera), either by prior experience (larvae of *Wyeomyia* mosquitoes), by use of keys to larvae of Chironomidae (Epler 2001), or (for the other families) according to the brief descriptions by Fish (1976). Although 27 years have passed since Fish (1976) reported his collections, the "*Neurosystasis*" (Psychodidae) and "*Stenomicra*" (Aulacigastridae) occurring in Florida bromeliads have not yet been formally described. G. J. Steck (FSCA) questioned the name *Neurosystasis* (he identified them as belonging to *Telmatoscopus*) and suggested contacting Larry W. Quate (Poway, California), a specialist in the family. Quate requested adult specimens reared from field-collected larvae in order to make a precise identification and, if necessary, prepare a formal description. Thereupon, JHF (with help from M. M. Cutwa and G. F. O'Meara, Florida Medical Entomology Laboratory, Vero Beach) obtained larvae from bromeliads in southeastern Florida and provided them to G. J. Steck who from them reared a few adults and shipped them to Quate in 1999-2000. Quate reported that they represent the first Nearctic record for a member of the genus *Alepia*, and he was pleased to see the associated larvae. Tragically, Larry Quate died in January 2002. It should be fairly easy to obtain more larval specimens and rear more adults to replace those unreturned by his estate.

Hymenoptera (ants identified by M. A. Deyrup)

Formicidae: *Camponotus floridanus* (Buckley) (2), *Camponotus planatus* Roger (26), *Crematogaster ashmeadi* Mayr (64), *Paratrechina longicornis* (Latreille) (50), *Pheidole megacephala* (Fabricius) (5), *Pheidole moerens* Wheeler (129).

Ichneumonidae: genus and species unidentified (2) (det. L. A. Stange, FSCA).

Aphelinidae: *Aphelinus* sp. (1) (see under Hemiptera and Homoptera).

Ant nests with brood were detected in *T. utriculata* (*C. planatus* and *P. longicornis*), *T. fasciculata* (*P. moerens*), and *T. setacea* (*C. ashmeadi*). The other ant specimens doubtless were foraging from nests elsewhere. It has long been known that ants will nest in the dry, outer leaf axils of bromeliads such as *T. fasciculata* and *T. utriculata* that hold water in their inner axils. One plant of *T. utriculata* in Sarasota provided space for nests of two species: *C. planatus* and *P. longicornis*. *Paratrechina longicornis*, *P. megacephala*, *P. moerens*, and *C. planatus* are adventive species.

Ants were identified from *Tillandsia* spp. in various Neotropical countries and Florida by Wheeler (1942). However, the *Tillandsia* were not identified to species level, nor were the localities in Florida nor dates of collection specified.

Table 2 arranges the collection data by sample number, with invertebrates identified to the level of family. This arrangement was designed to allow extraction of numerical data for statistical analysis. However, the Table suggested few patterns that would yield useful analysis. To further complicate the table by including species names would have been unwieldy.

A simple analysis was made by contrasting the content of the three smallest with three largest plants within each species (Tables 1 and 2), a valid statistical method. For *T. fasciculata* the smallest plants were nos. 17, 18, and 19 (with 17, 7, and 144 invertebrates). The three largest were 8, 9, and 20 (with 19, 18, and 36 invertebrates). The presence of an ant nest in plant 19, with 128 adult ants was the cause of the high count in a small plant. Even if all data for ants were omitted, the evidence for relationship of plant size to number of invertebrates would have been negligible.

For *T. utriculata*, the three smallest plants were 1, 3, and 4 (with 2, 16, and 10 invertebrates), and the three largest were 2, 16, and 24 (with 39, 226, and 135 invertebrates). In plant 24, ants accounted for 76 of the invertebrates. Whether or not we exclude data for ants, the largest plants clearly have more invertebrates, and these were mainly aquatic dipteran larvae (Ceratopogonidae, Culicidae, and Psychodidae; except in plant 24). If we exclude ants and aquatic insect larvae, the three smallest plants had 1, 14, and 9 invertebrates whereas the three largest had 17, 28, and 28; again there is a relationship between plant size and number of invertebrates, but it is shallower than when including the aquatics. If we include only the aquatic invertebrates, then the 3 smallest plants had 0, 2, and 1 invertebrates, whereas the three largest had 17, 191, and 31; the larger plants clearly had many more, but variance is huge. We might expect that the number of aquatic dipteran larvae would best be associated with volumetric capacity of bromeliad axils (calculated from length of longest leaf). But intraplant variance in numbers of contained invertebrates warns us that the fitting of regressions will suffer from high sums-of-squares errors. The presence of ant nests adds greatly to variance.

For the three *T. recurvata* plants sampled, the number of invertebrates was not related to plant size. For *T. setacea*, the three smallest plants were 5, 6, and 13 (with 11, 19, and 18 invertebrates) and three largest were 11, 12, and 14 (with 6, 18, and 4 invertebrates); there was no relationship of plant size to number of invertebrates.

Discussion and Conclusion

The total number of invertebrates in leaf axils of *T. utriculata* was related to plant size, but the number of aquatic insect larvae increased more strongly with plant size. The numbers of invertebrates were not or not clearly related to plant size in the other three bromeliad species, although such a relationship is something that would be expected given a very large number of samples (because larger plants provide more habitat). Data in Tables 1 and 2 could be the materials for hundreds of regression analyses, should anyone wish to do these.

This study scratches the surface of Florida's bromeliad fauna. It reaffirms that larvae of several aquatic Diptera (Psychodidae, Culicidae, Ceratopogonidae, Chironomidae, Muscidae, and Aulacigastridae), perhaps one species of scale insect (Ortheziidae), and perhaps one or more species of Lepidoptera (Tineidae and/or Gelechiidae) have an obligate relationship with bromeliads. The null hypothesis for all the remaining species is that they "just happened to be there" and may additionally be found in tree canopies or in leaf litter on the ground. This null hypothesis cannot now be tested for lack of studies of the canopy fauna or the leaf litter fauna in Myakka River State Park.

This in no way discounts the importance of bromeliads as habitat for large numbers of invertebrate species: how many other small plants have such a diversity of invertebrates? At least 70 families with 82 genera and 90 species are represented in the few (24) samples. Further sampling should yield very many more species (and genera and families) at least in Coleoptera, and perhaps some other orders, including species that just happen to be represented in the bromeliads at the time of sampling.

If sampling is to be repeated, this should be (a) with very many more samples to allow more replication and thus a more useful comparison between the faunas of the four bromeliad species, (b) with prior agreement (probably involving funded written subcontracts for expenditure of

time) from numerous specialist taxonomists to devote time to the project, (c) with the collector charged with rearing representatives of all the immature arthropods to the adult stage. The advantage of having more samples will be the availability of a series of adult specimens of every species represented, except perhaps a few of the transients. The advantage of rearing the immature arthropods will be that adult specimens will be available for identification, and identifiers will then have immature specimens reliably associated with the adults; thereafter, the specialists may be able to provide identification keys to the immature stages. The collector should be proficient in invertebrate classification, and should have the time to rear immature arthropods to the adult stage.

Raw data used to compile Table 2, on invertebrates associated with the 24 plants, will be offered to the "Bromeliad tank dwellers database" on the website of the Florida Council of Bromeliad Societies (http://www.fcbs.org). It records any animal species detected in or on a bromeliad, not just tank dwellers (the aquatic species in tanks). This could lead to detection of other animals frequently associated with bromeliads, even if it takes tens of thousands of records. It was not easy to obtain identifications to the species level of invertebrates collected from bromeliads in Florida, and we were only partially successful, and only for some groups. We warn investigators who would like to conduct similar studies in the Neotropics that they will encounter severe taxonomic problems. The effort to collect the specimens is small compared with the effort required to identify the specimens reliably to the species level. Identification not made to the species level is worth rather little. Taxonomists need to be convinced that the project is worth their support. In this project, some taxonomists obtained useful and interesting specimens, at least of *Xenylla* (an undescribed species), *Cerobasis guestfalica*, *Camplomyza* sp., *Alepia* (the first Nearctic record), and various mites of uncertain identity.

The sampling method did not collect microscopic aquatic organisms. For these, it would be better to use a siphon or large syringe (such as an "oven baster") to extract the water from leaf axils of *T. fasciculata* and *T. utriculata*, and to decant this water directly into Petri dishes for microscopic examination. Such a method should collect bacteria, Fungi Imperfecti, algae, rotifers, nematodes, platyhelminthes, annelids, ostracods and copepods. But identification of these would have been beyond the skills of the taxonomists involved in the present study.

Future projects of this nature in Florida with all four of these plant species are unlikely in the near future. This is because the weevil *Metamasius callizona* was detected in Myakka River State Park in September 1999 and, since then, has been relentlessly destroying the park's populations of *T. utriculata* and *T. fasciculata* (T. M. Cooper *in* Larson 2000). Similar destruction has been detected in almost all southern Florida counties. These two bromeliad species are rightfully listed in the Florida Administrative Code as endangered species. Recovery of their populations is unlikely unless the weevil can be brought under biological control.

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AUGMENTATION OF PARASITOIDS FOR BIOLOGICAL CONTROL OF CITRUS BLACKFLY IN SOUTHERN TEXAS

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ABSTRACT

Two parasitoid species, *Amitus hesperidum* Silvestri and *Encarsia opulenta* (Silvestri), were released in an augmentative program to control citrus blackfly, *Aleurocanthus woglumi* Ashby, in the citrus growing areas of southern Texas. Releases were made with laboratory-reared and field insectary parasitoids. Six citrus groves were closely monitored, and evaluations made during and after releases suggested that both parasitoid species became reestablished and exerted control over pest populations. Dissection of citrus blackfly immatures led us to suggest that *E. opulenta* increased in larger numbers than *A. hesperidum*, and that a stable host-natural enemy relationship became established.

Key Words: *Aleurocanthus woglumi*, *Amitus hesperidum*, *Encarsia opulenta*, augmentative biological control

RESUMEN

Dos especies de parasitoides, *Amitus hesperidum* Silvestri y *Encarsia opulenta* (Silvestri) fueron liberadas en un programa de aumentó para el control de la mosca prieta de los cítricos. *Aleurocanthus woglumi* Ashby, en áreas donde siembran los citricos en el sur de Texas. Las liberaciones fueron hechas usando parasitoides criados en el laboratorio y los del insectario del campo. Un monitoreo preciso de seis huertos de cítricos fue hecho, y las evaluaciones hechas durante y después de la liberación que sugerieron que ambas especies de parasitoides se re-establecieron y ejercieron un control sobre la población de la plaga. La disección de inmaduras de la mosca prieta de los cítricos tambien sugerió que el *E. opulenta* se aumento en números mas altos que el *A. hesperidum*, y que una relación estable entre el hospedero y el enemigo natural fue establecida.

The citrus blackfly, *Aleurocanthus woglumi* Ashby, first invaded the Lower Rio Grande Valley of Texas in 1955 on residential citrus (Smith et al. 1964), and again near Brownsville in 1971 in both residential citrus and commercial groves (Hart et al. 1973). An augmentative biological control program to establish parasitoids was initiated in 1974 with release of three species, *Amitus hesperidum* Silvestri (Hymenoptera: Platygasteridae), *Encarsia* (= *Prospaltella*) *opulenta* (Silvestri), and *E. clypealis* (Silvestri) (Hymenoptera: Aphelinidae). These releases were made from laboratory-reared and field-collected cultures in Mexico (Hart 1978). Evaluations undertaken from 1977-1982 indicated a widespread distribution of *E. opulenta*, but few *A. hesperidum*, and no *E. clypealis*, suggesting local competitive displacement by *E. opulenta* in groves with effective parasitoid regulation (Summy et al. 1983).

Citrus blackfly population densities remained stable under excellent biological control until the mid 1980s (Summy et al. 1983). Following a severe freeze in December 1983, citrus blackfly densities surged while concomitant parasitoid densities apparently remained low. Citrus blackfly populations reached damaging levels during the 1988 and 1989 seasons, especially in central valley groves (French et al. 1990). A biological control program of parasitoid augmentation was initiated in June 1989, and results suggested reestablishment of *E. opulenta* and *A. hesperidum* (Meagher et al. 1991). However, another severe freeze in December 1989 halted evaluation efforts.

Although most commercial groves had no citrus blackfly populations after the freeze (unpublished data), populations were discovered in citrus nurseries and on residential citrus trees in early 1990 (French & Meagher 1992). Newly planted and commercial groves located near residential areas soon were infested with dense, largely unparasitized citrus blackfly populations (unpublished data). An augmentation program was initiated in 1992 to increase biological control efficacy in commercial citrus groves. This was accomplished by releasing parasitoids into residential citrus so that "field insectaries" could be developed. Commercial groves were then sampled with yellow sticky traps (Harlan et al. 1979; Summy et al. 1986) and leaf observation, so that groves containing large citrus blackfly densities

could be identified as release sites. The final step was to transfer laboratory- and field insectary-produced parasitoids into infested groves. This report describes the seasonal progression of the host and its natural enemies in commercial citrus groves in southern Texas.

Materials and Methods

Groves Sampled

Grapefruit and orange groves selected for this study were located throughout the Lower Rio Grande Valley in both Cameron and Hidalgo counties. They included groves near Bayview (10 ha., 'Rio Red' grapefruit, sampled 21 July 1992-21 March 1995; 7.2 ha., 'Marrs' and 'Valencia' orange, sampled 23 November 1993-21 March 1995), Donna (16.2 ha., 'Ruby Red' and 'Star Ruby' grapefruit, 27 July 1992-22 February 1994), Mercedes (16.2 ha., 'Ruby Red' grapefruit and 'Valencia' orange, 7 December 1993-21 March 1995), Edinburg (8.3 ha., 'Ruby Red' grapefruit, 22 July 1992-12 April 1994), Mission-grapefruit (12.1 ha., 'Rio Red' grapefruit, 15 July 1992-22 March 1994), and Mission-orange (3.4 ha., 'Marrs' orange, 15 July 1992-22 March 1994).

Parasitoid Releases

Augmentation of both *A. hesperidum* and *E. opulenta* was accomplished with laboratory-produced and field-collected specimens from Florida (Florida Department of Agriculture, Division of Plant Industry, Gainesville) and field insectary-produced parasitoids from Texas. Both the cup and paper bag methods of French et al. (1990) and Meagher et al. (1991) were used to disperse parasitoids. From January 1992-February 1993, over 92,000 *A. hesperidum* and 18,000 *E. opulenta* were released throughout the citrus growing region, and selected releases of both species of parasitoids were made later during 1993.

Citrus Blackfly Sampling

Citrus blackfly infestation and parasitization levels were sampled by two techniques. First, the percentage of leaves infested with citrus blackfly was determined by examining four branches of eight trees. The total number of leaves and number with live citrus blackfly immature stages were recorded. Selection of each branch was based on directional orientation (quadrants: southeast, southwest, northwest, northeast). Five of the trees were 'station' trees that were sampled each time; an additional three trees were selected randomly each sample date. This sampling technique was not conducted in the Bayview-orange grove. Analysis of variance (PROC GLM, LSD mean separation test, SAS Institute 1995) was used to examine variation among trees or among quadrants. Parasitization was calculated by dissecting and examining a subsample of at least 100 fourth stage nymphs ('pupae').

Beginning in late 1993, an additional sampling technique was developed to provide a closer examination of citrus blackfly parasitization. These samples were taken in the grapefruit and orange sections of the Bayview grove and in the Mercedes grove. From each tree, one hundred leaves that contained citrus blackfly pupae were collected. From this collection, ten leaves containing pupae were selected and returned to the laboratory for dissection. Each pupa was categorized as live, dead, or emerged citrus blackfly; or live, dead, or emerged parasitoid. Dead citrus blackfly and parasitoids were represented by desiccated remains. Emerged individuals were represented by either the characteristic pupal exuvia split by citrus blackfly or circular exit holes created by parasitoids. Parasitization by species was not identified, although exit hole numbers per pupa at times provided species information. Generally, two exit holes indicated the presence of *A. hesperidum*, although rarely an individual larva of both species was found live in one host pupa. Parasitoid adults searching on leaves were noted during sampling.

Results

Citrus blackfly-infested leaves exhibited significant inter- and intra-tree variation when each grove was analyzed individually ($P < 0.0001$; $P < 0.003$, respectively). The northwest quadrant of the tree always contained more infested leaves than the southeast quadrant (Table 1). These results are in agreement with previous studies in Florida and Texas which suggested high inter-tree variation (Dowell & Cherry 1981), although Gilstrap et al. (1980) found more citrus blackfly in the northwestern quadrant of the tree. Although our sampling described clear quadrant differences in population level, we agree with other researchers that collection of leaves for population sampling or parasitoid efficacy should be from all tree areas, especially when pest densities are low (Cherry & Fitzpatrick 1979; Gilstrap et al. 1980).

The Donna, Edinburg and two Mission grove locations selected for monitoring in 1992 had initial citrus blackfly populations below 10% infested leaves (Fig. 1). Leaf samples from these groves showed maximum citrus blackfly infestations ranging from 44.5 to 82.1%, but leaf samples taken at the end of sampling showed little to no citrus blackfly infestation (Table 2). Parasitization from both species ranged from 62.1-100% in these groves, but by the end of sampling in 1994 was much reduced due to the scarcity of hosts.

Population dynamics of host and parasitization in the Bayview grove showed low numbers of

TABLE 1. PERCENT CITRUS BLACKFLY-INFESTED LEAVES BY TREE QUADRANT FROM CITRUS GROVES IN THE LOWER RIO GRANDE VALLEY, TEXAS, 1992-1995.

Grove	*df*	F	Tree quadrant			
			Northwest	Northeast	Southwest	Southeast
Bayview	3, 1271	7.2	18.2 ± 1.5 a	15.3 ± 1.4 b	16.0 ± 1.4 ab	12.2 ± 1.3 c
Donna	3, 775	30.1	19.9 ± 1.7 a	17.9 ± 1.7 a	13.3 ± 1.3 b	8.4 ± 1.1 c
Mercedes	3, 491	5.6	81.1 ± 2.2 a	80.7 ± 2.2 a	77.9 ± 2.6 a	73.0 ± 2.8 b
Edinburg	3, 759	21.0	23.1 ± 1.9 a	20.2 ± 1.8 b	19.9 ± 1.7 b	12.8 ± 1.4 c
Mission-grapefruit	3, 775	4.9	32.7 ± 2.5 a	31.8 ± 2.5 a	31.1 ± 2.5 a	28.8 ± 2.4 ab
Mission-orange	3, 806	3.1	19.2 ± 1.7 a	17.9 ± 1.6 ab	15.7 ± 1.6 b	15.8 ± 1.6 b

Means (± SE) within the same row followed by the same letter are not significantly different, LSD ($P > 0.05$).

citrus blackfly initially when sampled in summer 1992 (Fig. 2). Citrus blackfly populations rose to over 60% leaves infested by late fall 1993, until increasing parasitization appeared to reduce host populations. A closer examination of grapefruit trees showed proportionally high levels of para-

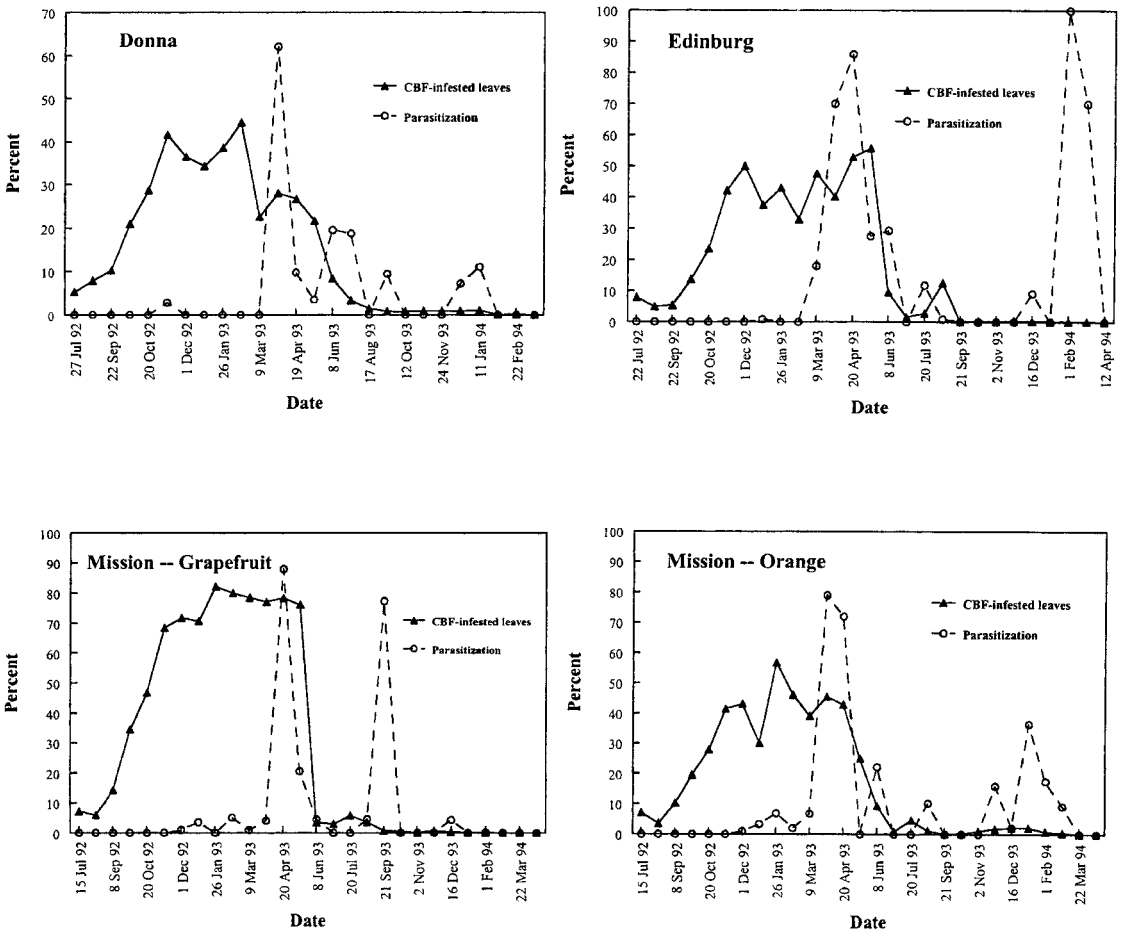

Fig. 1. Population densities of citrus blackfly (percent leaves infested) and parasitization (percent fourth stage parasitized) due to *Amitus hesperidum* and *Encarsia opulenta*, in three Lower Rio Grande Valley, Texas citrus groves, 1992-1994.

TABLE 2. HIGHEST AND FINAL PERCENT CITRUS BLACKFLY-INFESTED LEAVES AND PERCENT PARASITIZATION BY *E. OPULENTA* AND *A. HESPERIDUM* FROM GRAPEFRUIT AND ORANGE GROVES IN THE LOWER RIO GRANDE VALLEY, TEXAS, 1992-1995.

Grove	Highest infested leaves (%)	Highest parasitization (%)	Final infested leaves (%)	Final parasitization (%)
Bayview	65.5 ± 5.9	79.3	2.8 ± 0.5	48.7
Donna	44.5 ± 5.4	62.1	0.03 ± 0.03	0
Mercedes	100.0 ± 0	92.9	68.1 ± 3.1	59.2
Edinburg	55.7 ± 4.3	100.0	0	0
Mission-grapefruit	82.1 ± 2.5	88.0	0	0
Mission-orange	56.8 ± 5.2	79.0	0	0

sitization occurred during late winter 1993 and spring 1994, with over 50 immature parasitoids per leaf found in the 15 February, 29 March, and 19 April samples, and 65 per leaf in the 20 June sample (Fig. 3 a, b). Adults from many of the parasitized pupae during this period had already emerged. Adult *A. hesperidum* was the predominate species observed on leaves during early sampling, and although *E. opulenta* adults were present, their numbers did not increase until fall 1994. The highest citrus blackfly population density also occurred during spring and summer 1994, with a peak of 109.7 ± 30 live immatures found in the 10 May sample (Fig. 3a). The grower, without our recommendation, applied an unknown insecticide in February and May, resulting in mortality of both citrus blackfly and parasitoids (20.7 ± 6.8 dead parasitoid immatures per leaf, 20 June sample) (Fig. 3b). Populations of citrus blackfly, *A. hesperidum*, and *E. opulenta* all declined after the 20 June sample. Samples taken in early 1995 showed low populations levels of the host, but active populations of both parasitoids species.

Overall population density of citrus blackfly was lower in the Bayview oranges, peaking at 26.7 ± 8.4 live immatures per leaf in late 1994 (Fig. 3c). As in the grapefruit, high mortality occurred during June through August as a result of an insecticide application. However, both citrus blackfly and parasitoid activity increased in the late fall. Over 10 live or emerged parasitoids per leaf were found by the end of the study, including both *A. hesperidum*, and *E. opulenta* (Fig. 3d).

Sampling in the Mercedes grove showed initial citrus blackfly populations already close to 40% infested leaves (Fig. 2). This grove was the only one that had high levels of citrus blackfly at the conclusion of sampling, although parasitization was also high (Fig. 2). Intensive sampling in late 1993 through early 1994 showed medium levels of live and dead citrus blackfly, with low levels of parasitization (Fig. 4a). By spring, citrus blackfly populations increased to >100 live pupae per leaf in May. Populations remained high through summer and early fall, peaking with an average of 120.3 live and 3.8 emerged citrus blackfly pupae per leaf in October 1994. However, parasitoid activity was increasing by August, as exemplified by observation of searching adult *E. opulenta* on leaves. Parasitization increased through fall and into 1995, with >200 live or emerged parasitoid immatures found in the 1 November sample (Fig. 4b). Citrus blackfly populations decreased after the 22 November sample, and from the 19 December sample to the conclusion of the study, over 70 live or emerged immature parasitoids (predominately *E. opulenta*) per leaf were documented.

DISCUSSION

Release of these and other exotic parasitoid species against citrus blackfly in Mexico during the early 1950s formed the resource for future releases in the Western Hemisphere. By the end of 1953, over 300 million adults (242 million of *A. hesperidum* alone) were dispersed in Mexico (Flanders 1969). The discovery of the pest in Ft. Lauderdale, FL residential citrus during January, 1976 led to the release of *A. hesperidum*, *E. opulenta*, and *E. clypealis* from laboratory cultures in General Teran, Mexico (Hart et al. 1978). Since then, over 250,000 parasitoids from laboratory colonies, field collections, and movement of infested and parasitized citrus leaves were released in Florida from October 1979 through May 1980 (Nguyen et al. 1983). Laboratory cultures and field collections from Florida formed the basis for the material that was augmented into Texas citrus.

Our results suggest the successful establishment of *A. hesperidum* and *E. opulenta* in the Texas citrus agroecosystem following severe freezes, and the reduction of citrus blackfly populations. Insecticide applications, especially within the grapefruit trees at Bayview, limited our ability to follow natural enemy interactions. However, postbloom and summer selective pest management chemical sprays have been shown to have only short term influence on citrus blackfly natural enemy populations (Fitzpatrick et al. 1978, 1979).

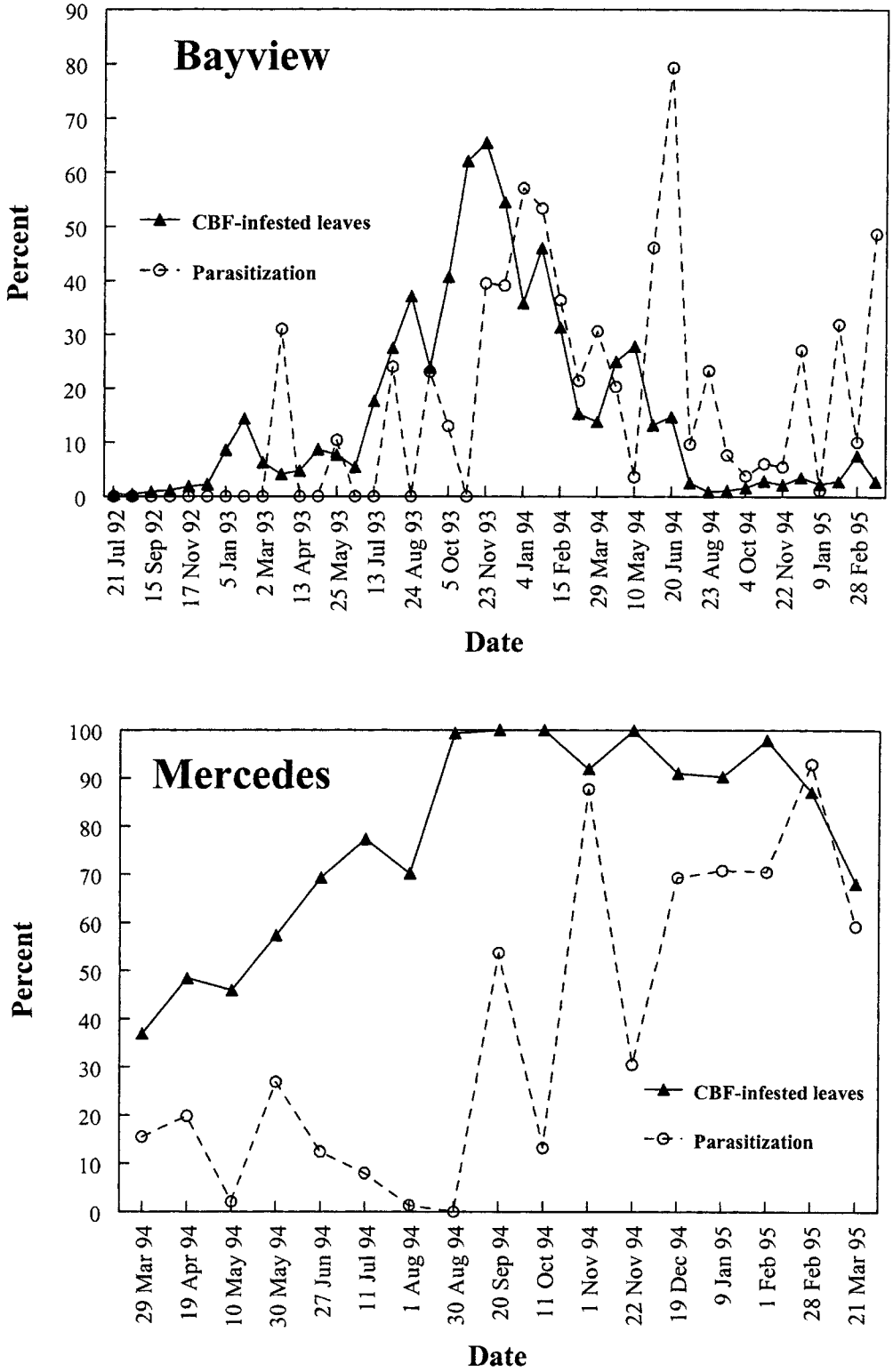

Fig. 2. Population densities of citrus blackfly (percent leaves infested) and parasitization (percent fourth stage parasitized) due to *Amitus hesperidum* and *Encarsia opulenta*, in two Lower Rio Grande Valley, Texas citrus groves, 1992-1994.

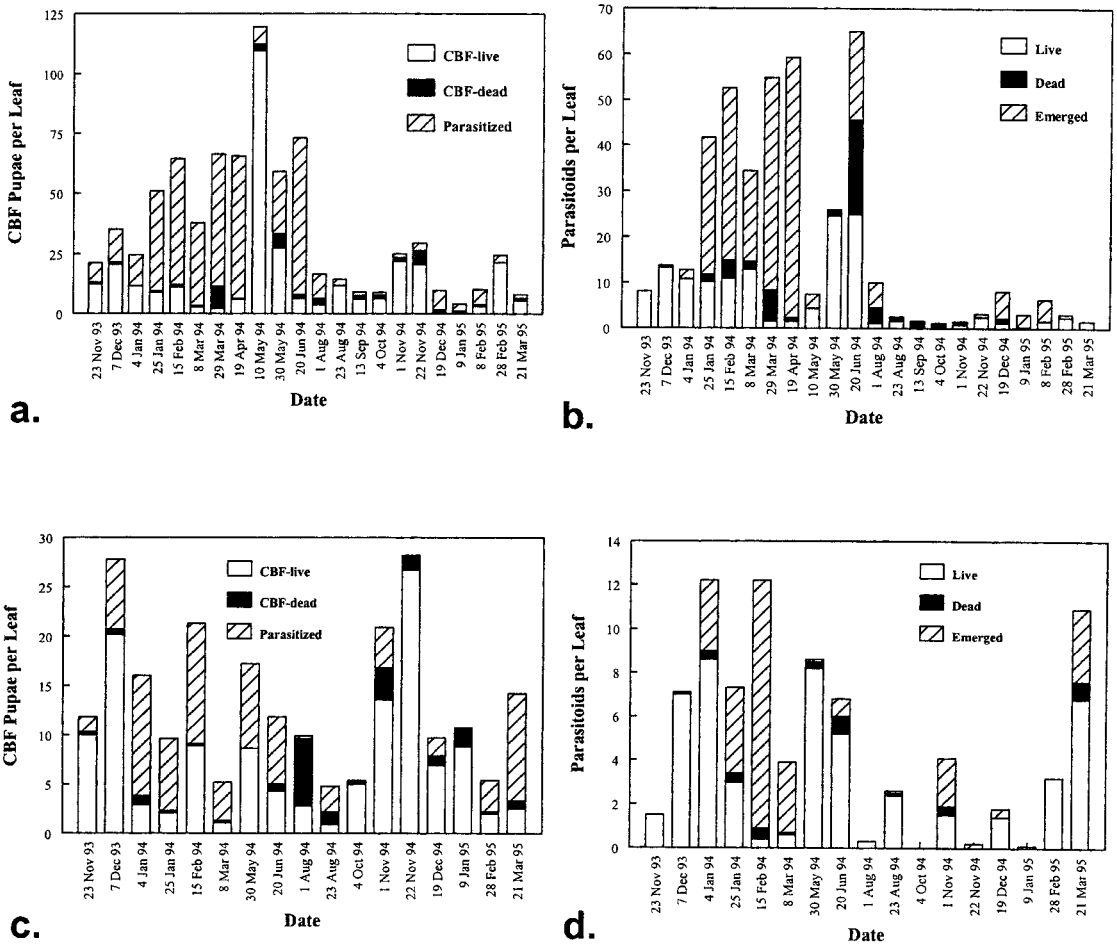

Fig. 3. Population densities of citrus blackfly and two parasitoid species in a grapefruit (a, b) and orange (c, d) grove, Bayview, Texas. Bars correspond to live, dead, or parasitized citrus blackfly per leaf (a, c), or live, dead, or emerged parasitoids per leaf (b, d).

In the Mercedes grove, results suggested increasing populations of *E. opulenta* and population suppression of citrus blackfly. Several reports have documented population regulation by this parasitoid, even within groves under pest management chemical applications (Cherry & Pastor 1980; Swezey & Cano Vasquez 1991). *Encarsia opulenta* has been shown to be able to competitively displace populations of other *Encarsia* species and *A. hesperidum* because of its ability to maintain a stable interaction with its host under low host populations due to density-dependent searching of adult parasitoids (Summy et al. 1983, 1985). In a laboratory study, *E. opulenta* females showed preferences for citrus blackfly that were previously parasitized by *A. hesperidum* (Dowell et al. 1981), although *A. hesperidum* larvae can avoid predation by *E. opulenta* larvae by "hiding" in the midgut (Flanders 1969).

This report suggests that citrus blackfly populations were reduced in groves selected for parasitoid augmentation. Parasitoid populations in these groves increased temporally either due to our reestablishment program or due to the increase of naturally-occurring populations that survived the freeze in residential and commercial citrus trees. Since we did not determine if parasitism was attributed to naturally-occurring or released individuals, the role of our augmentation program on parasitoid reestablishment cannot be identified explicitly. Only carefully planned experiments comparing parasitoid populations in "control" and "treated" groves with similar residential and commercial citrus habitats will provide this information. This type of experimentation has not been accomplished on a large scale in studies involving citrus blackfly biological control because of grower, citrus industry, and logistical demands.

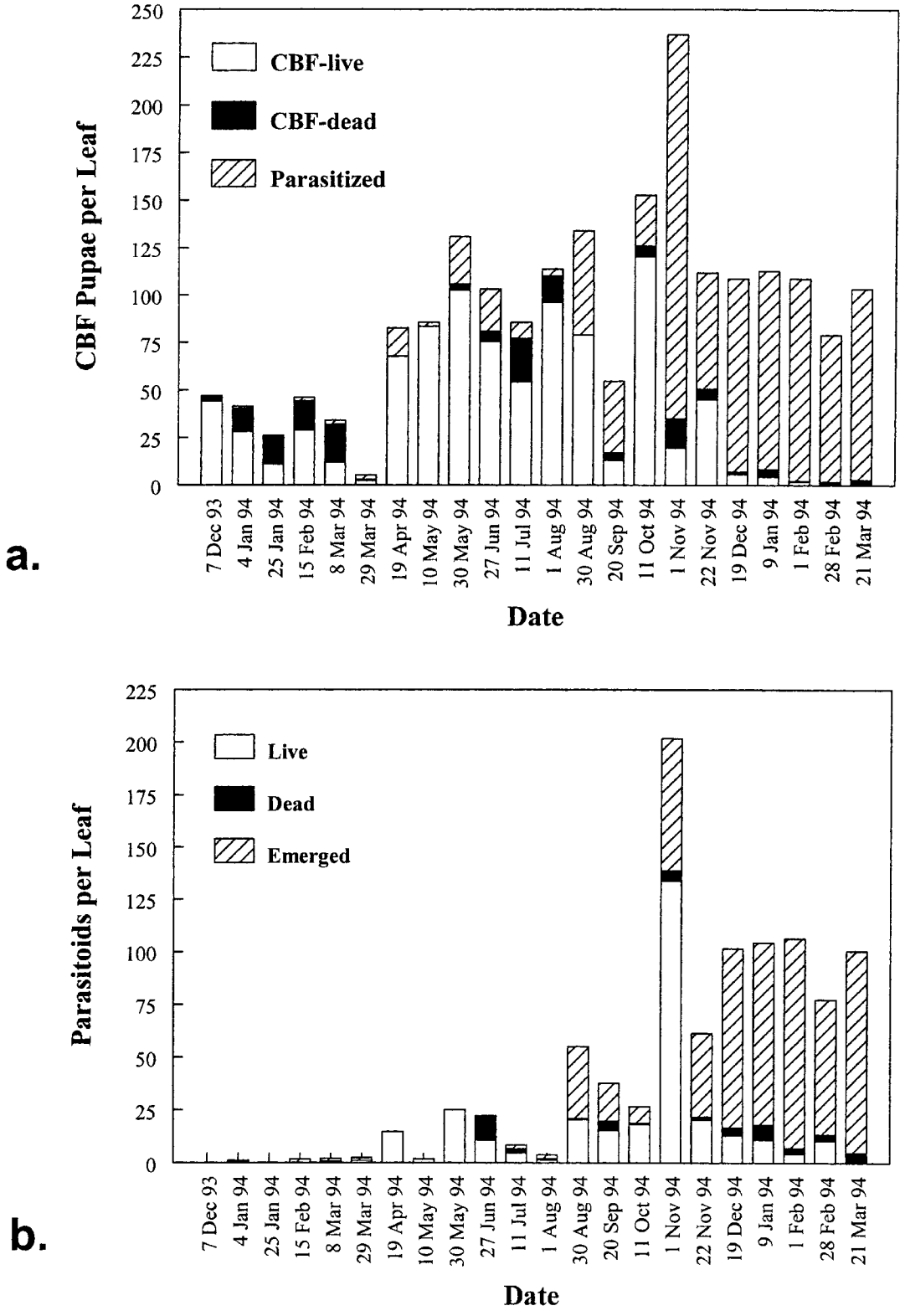

Fig. 4. Population densities of citrus blackfly and two parasitoid species, in a citrus grove, Mercedes, Texas. Bars correspond to live, dead, or parasitized citrus blackfly per leaf (a), or live, dead, or emerged parasitoids per leaf (b).

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AN ARTIFICIAL DIET FOR THE BUTTERFLY *PHYCIODES PHAON* (LEPIDOPTERA: NYMPHALIDAE)

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ABSTRACT

We reared newly hatched Phaon Crescent butterfly larvae to the adult stage on a completely artificial diet. About 37% of first instars survived to the adult stage. Addition to the diet of freeze-dried host plant leaves equal to 10% by weight of dry ingredients produced up to 66% survival to the adult stage. Survival of larvae and production of adults on the artificial diet without host plant leaves was increased to equal that of diet with host plant leaves by adding 5% glucose or 5% Beck's salt mix. Although the ovaries of females produced on host-free artificial diet appeared to be mature at emergence and contained mature-looking eggs, we never obtained viable eggs from them. In contrast, females produced on the artificial diet containing at least 10% by weight of freeze-dried host plant leaves laid viable eggs, and four successive generations were reared on the artificial diet with 10% freeze-dried host plant leaves. Males produced on the artificial diet without host plant tissue displayed abnormalities in the shape of the testes and parts of the vas deferentia, compared to males reared on the diet with freeze-dried host leaves or on living host plants. The role of host plant tissue in nutrition and reproduction of both male and female Phaon crescents remains to be determined.

Key Words: artificial diets, butterflies, Phaon crescent, Nymphalidae, insect-plant interaction, *Phyla nodiflora*, ovary, testes

RESUMEN

Nosotros criamos larvas de la mariposa cresciente Phaon recién eclosionadas hasta la estadia adulta sobre una dieta artificial. Aproximadamente, 37% de las larvas de la primera estadia sobrevivieron hasta la estadia adulta, pero los adultos no se aparearon ni reproducieron. La adición de hojas congeladas y secadas de la planta hospedera a la dieta igual a 10% del peso seco de los ingredientes produjieron una sobrevivencia de 66% hasta la estadia adulta, y los adultos se aparearon y pusieron huevos que eclosionaron. La sobrevivencia de las larvae y la producción de adultos sobre una dieta artificial sin hojas de la planta hospedera fue aumentada para ser igual que la sobrevivencia con hojas de la planta hospedera por añadir 5% de glucosa o 5% de la mezcla de sal de Beck, pero los adultos no se aparearon a menos que la dieta tenia hojas de la hospedera. Los ovarios de las hembras producidas sobre una dieta artificial sin la planta hospedera aparecieron ser maduras al emergir y tenian huevos maduros, igual como los ovarios de las hembras criados sobre la planta hospedera. Ningún abnormalidad en las estructuras reproductivas internas de las hembras producidas sobre la dieta artificial fue detectada. Los machos producidos sobre la dieta artificial sin el tejido de la plant tenian abnormalidad en las estructuras reproductivas internas. Asi, ambos sexos criados sobre una dieta artificial evidentemente tienen algunas abnormalidad funcionales para prevenir el apareamiento y el éxito reproductiva. El papel del tejido de la planta hospedera en influenciar el comportamiento y la reproducción de los crescientes Phaon es desconocido.

Many moths, beetles, crickets, grasshoppers, and other insects, but only two or three butterfly species, can be reared on artificial diets (Singh 1977). The cabbageworm butterfly (*Pieris rapae* (L.)) (Webb & Shelton 1988) and the painted lady butterfly (*Vanessa cardui* (L.)) have been reared on artificial diets. Semiartificial diets that contain host-plant material have been published for rearing the Monarch butterfly. The need to study and control pest insects probably has contributed to the development of artificial diets for many insects, but most butterflies are not pests on economic crops and little effort has been devoted to developing artificial rearing media for them. Butterflies tend to be restricted to one or only a few host plants as larvae, and possibly they are very sensitive to the balance of nutrients and/or presence of specific feeding cues in their host plants. A practical difficulty in working with butterflies is that many are active only part of the year, and their larval host plants are often seasonal.

The Phaon crescent, *Phyciodes phaon* (Edwards), is a small butterfly present over much of the southeastern United States. A number of factors make the Phaon crescent a suitable butterfly for study, including year-round distribution of the butterfly and its host plant in Florida. The adults do not diapause, and they mate and lay eggs in

the laboratory. Females lay their eggs on the underside of leaves of the host plant *Phyla nodiflora* (L.) Greene in the family Verbenaceae (Minno & Minno 1999; Emmel & Kenny 1997; Genc 2002). In preliminary trials with several published diets for rearing butterflies and moths, Genc (2002) found that the only diet formulation that allowed a few adults to be produced was the pinto bean (PB) diet developed for certain moths (Guy et al. 1985). Survival on the PB diet was poor, however, and adults produced did not mate. Our objectives in this paper are to describe (1) diets that improve survival of Phaon crescent larvae and adult production, (2) diets that promote mating and reproduction of adults, and (3) female and male internal reproductive systems of Phaon crescents reared on completely artificial diet with those reared on the host plant.

Materials and Methods

The Butterfly and Host Plant

A colony of *Phyciodes phaon* was started from females collected on the University of Florida campus. The host plant was collected from the campus and vicinity, and maintained in containers and small outdoor plots. Leaves of the host plant were frozen in liquid nitrogen, ground while frozen in a mortar, and freeze-dried. The freeze-dried host plant leaves were stored at -20°C until needed. Adults were allowed to lay eggs on the leaves of the living host plant, and newly hatched first instars were removed and placed on diets. A breeding colony of the butterfly was maintained in the laboratory on host plants, and adults were provided with 10% honey in water.

Preparation of the Pinto Bean Diet from Components

The pinto bean (PB) diet was prepared from individual components purchased from BioServ (One 8th Street, Frenchtown, NJ 08825, USA). We mixed pinto bean meal (19 g), wheat germ (14 g), torula yeast (8 g), casein (7 g), gelcarin (3 g), methyl paraben (0.5 g), and sorbic acid (0.3 g) and added the mixture to 182 ml cold water with stirring by a mechanical mixer. The aqueous mixture was heated slowly (requiring about 15 minutes) on a hot plate to 70°C with continuous stirring. Formaldehyde (1 ml) was added and mixing was continued for about 3 minutes without further heating. Ascorbic acid (0.9 g) was added and mixing was continued an additional 3 minutes without heating, and finally tetracycline (0.01 g), BioServ Vitamin Mix #F8095 for Lepidoptera (0.8 g), and propionic acid (0.3 ml) were added with additional mixing for 2-3 minutes. The mixture was poured into paper cups, allowed to cool and gel at room temperature, and stored in a refrigerator until needed. When ground, freeze-dried plant leaves were added to the pinto bean diet, addition was made with the vitamin mixture to minimize heat damage to the host plant material.

Diet Modifications

Diets were formulated by incorporating 1% glucose, 5% glucose, 1% Beck's salt mixture, 5% Beck's salt mixture, 1%, 5%, 10%, or 20% ground, freeze dried host plant leaves into the PB diet. Diets were tested by placing 25 newly hatched larvae on each of 3 replicates of each diet. The criteria for evaluating a diet were number of adults reared and whether the adults mated and females laid viable eggs.

Dissection

The abdomen of adults was brushed with a camel's hair brush dipped in 70% ethyl alcohol to remove scales, and then opened ventrally from the first to the terminal abdominal segment. The terminology used by Dong et al. (1980) was used to describe the internal reproductive structures.

Statistics

For comparing the number of adults produced on modifications of the PB diet, we used binary logistic regression analyses (Harrell 2001; Hosmer & Lemeshow 2000). Chi Square tests were used to determine the statistical significance of the model parameters and an overall Chi Square test assessed the hypothesis of no overall treatment difference. When a significant Chi Square value was obtained, the means for adult production on each tested diet were transformed from non-linear function to linear function and least square estimates of the diet-specific probabilities, P, of survival to the adult stage were obtained by inverting the log odds model.

Results

Diet Modifications

Survival to the adult stage was statistically higher on PB diet with 10% or 20% freeze-dried host plant leaves than with only 1% or 5% leaves (Table 1). Moreover, adults from the diets containing 10% and 20% leaves reproduced and enabled us to maintain a colony, but adults produced with 1% or 5% leaf tissue in the PB diet did not reproduce. Addition of 5% glucose or 5% Beck's salt mix to the PB diet produced adults in numbers statistically equal to numbers of adults produced with 10% host plant leaves in the PB diet, but none of the adults from glucose or salt modified diets reproduced. Numbers of adults produced on diets with 1% host leaves, 5% host leaves, 1% glucose, 1% Beck's salt mix, or the original PB formula were not statistically different from each other.

TABLE 1. PRODUCTION OF ADULTS ON PB DIET OR PB DIET WITH AN AMENDMENT. NEWLY HATCHED LARVAE (25) WERE PLACED ON EACH OF THREE REPLICATES OF EACH DIET.

PB diet + Amendment	Mean (± SD) number of adults produced per replicate	Percent adults
1% host plant leaves	8.0 ± 0.0 a	32
5% host plant leaves	12.0 ± 1.4 a	48
10% host plant leaves	16.5 ± 0.7 b	66
20% host plant leaves	13.5 ± 2.1 b	54
PB diet	8.5 ± 0.7 a	34
1% glucose	10.0 ± 1.4 a	40
5% glucose	17.5 ± 2.1 b	70
1% Beck's salt mix	11.5 ± 0.7 a	46
5% Beck's salt mix	16.5 ± 0.7 b	66

Values in a column followed by the same letter are not significantly different from each other at $\alpha = 0.01$ level.

Anatomy of Phaon Crescent Internal Reproductive Structures

The structure of the internal reproductive system of a 10-day-old female produced on the host plant is shown in Figure 1A and the ovary of a newly emerged female adult from the PB diet is shown in Figure 1B. Adult females produced on both food sources appeared to have mature or nearly mature eggs in the terminal follicles of each ovariole at emergence, with four ovarioles in each of two ovaries. Although a large amount of fat body associated with the ovaries makes counting individual egg chambers very difficult, one newly emerged female was determined to have approximately 49 egg chambers in each ovariole. Not enough observations were made, however, to determine an average for number of egg chambers per ovary or eggs laid. The lateral oviducts guide eggs to a common, medial oviduct leading to the genital chamber (the bursa copulatrix). Paired lateral accessory glands are each connected to the median oviduct.

The structure of the internal reproduction organs from a male produced on the host plant is shown in Figure 2A, and those from a male produced on the PB diet is shown in Figure 2B. Fused testes form one testicular body in males. The testicular body is round and dark reddish brown in males produced on the host plant and on PB diet

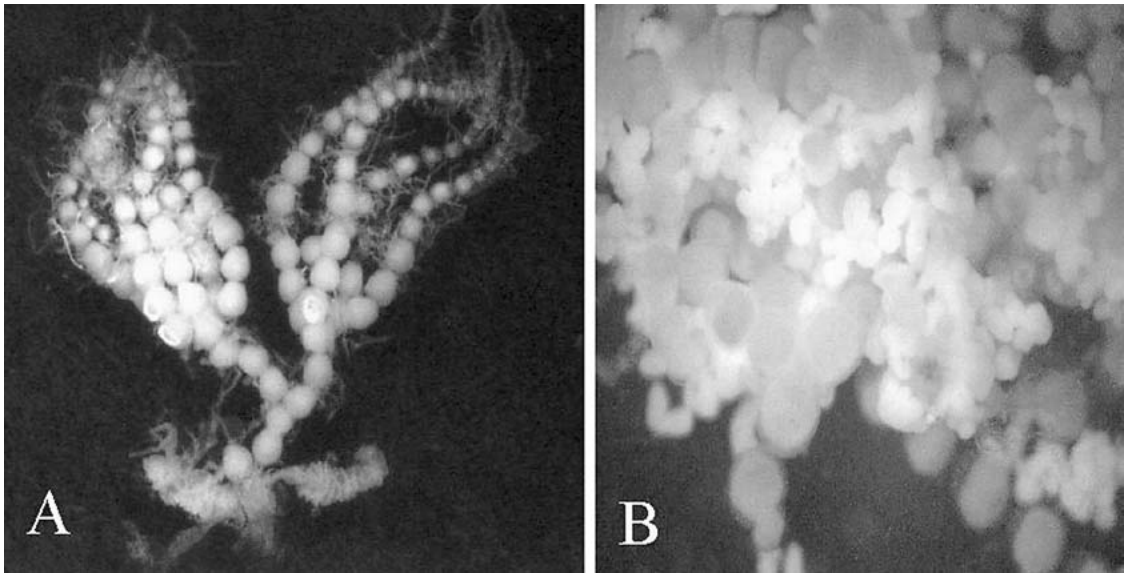

Fig. 1. Internal reproductive structure of *Phyciodes phaon* female. A. The ovary was dissected from a 10-day-old female reared on the host plant. The fat body has been almost entirely used up in production of eggs. The four ovarioles per ovary, and individual egg chambers can be seen. B. Ovary dissected from a newly emerged female produced on PB diet. Mature-looking eggs are present surrounded by large amounts of fat body, which is characteristic of newly emerged females produced on host plant, PB diet with 10% leaves, or PB diet.

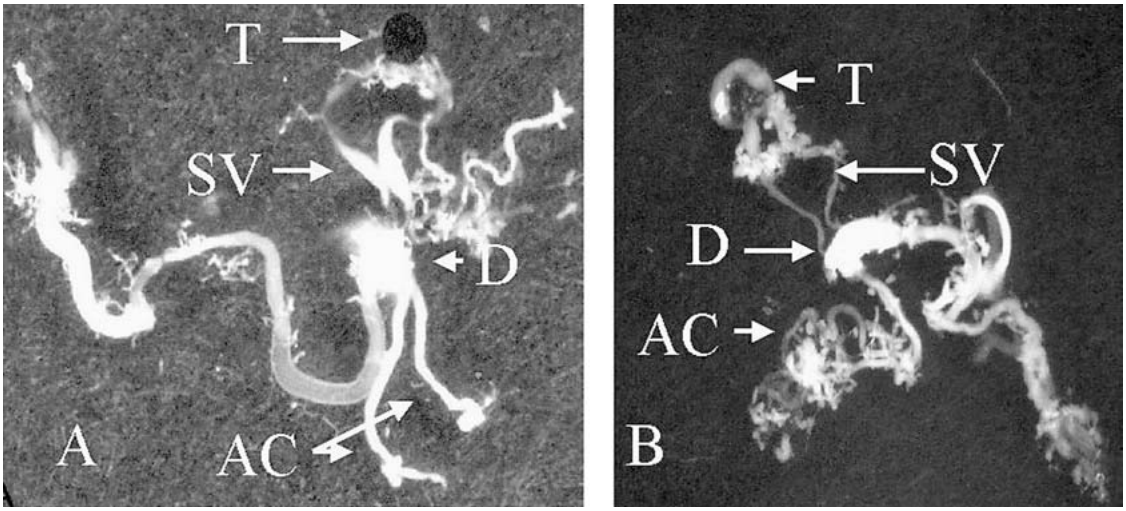

Fig. 2. Internal reproductive structures of *Phyciodes phaon* male. A. Internal structures dissected from a newly emerged male produced on the living host plant. The fused testes (T) seminal vesicle (SV) leading to the ductus ejaculatorius duplex (D) and accessory glands (AC). The duplex loops join to form the ductus ejaculatorius simplex leading to the aedeagus. B. The photograph shows the fused testes and related internal structures from a newly emerged male reared on PB diet. Note larger, discolored testes (T) and atrophied seminal vesicles (SV).

with 10% freeze-dried leaves. In males produced on the PB diet, the testicular body is not uniformly colored as in males from the host plant. There are differences also in the appearance of the vas deferentia of the males. The vas deferentia of males produced on the host plant or PB diet with 10% freeze-dried leaves have swollen vas deferentia near the midlength, forming the seminal vesicles. The seminal vesicles of males produced on the PB diet are not swollen and appear to be atrophied.

Discussion

The PB diet designed for moths clearly is not satisfactory as a diet for the Phaon crescent. As originally formulated, it allows only about 37% of newly hatched larvae to become adults, and the adults do not reproduce. Thus, a colony cannot be maintained on the artificial diet. We improved the diet with respect to both survival and ability of adults to reproduce by adding freeze dried host plant leaves equal to 10% by weight of dry ingredients of the PB formula. This improved diet produced from 66% up to 78% adults in some experiments from first instars started on the diet, and the adults mated and reproduced, maintaining the colony. Although we also improved the PB diet to give good production of adults by addition of 5% glucose or 5% Beck's salt mix, the adults did not reproduce. Glucose in the PB formula may be a feeding stimulant, and/or a readily available carbohydrate energy source. The original PB formula does not include a simple carbohydrate, nor does it include a salt mixture. Lepidopterans, most of which are phytophagous, typically have a relatively high K^+/Na^+ ratio in the hemolymph, in contrast to omnivorous and some carnivorous feeders which have low K^+/Na^+ ratios. Beck et al. (1968) developed a salt mixture (now sold as Beck's salt mixture) relatively high in K^+ and Mg^{2+} and low in Na^+ and Ca^{2+} and showed that it improved the growth and survival of the European corn borer, *Ostrinia nubilalis* (Hübner). Wesson's salt mix often has been used in insect diets (Singh 1977), but it was developed for vertebrate animals, and it has high Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios suitable for vertebrates. Although it works for some insects, probably it is not optimal for phytophagous insects.

Dethier (1954) and Fraenkel and Blewett (1943) emphasized that host plant selection is determined by the presence or absence of nonnutritive secondary plant substances that act as feeding deterrents or stimulants. Various imbalances of the nutrients in a diet can stress insects, and reduce growth and survival (House 1965; House 1969). The small amount of host leaves in the artificial diet may aid digestion and assimilation of nutrients, and may help balance some of the nutrients in the PB diet formula.

Newly emerged females reared on the host plant and on the PB diet with added host plant leaves have mature ovaries with apparently mature eggs at the time of emergence. In this respect they are similar to the cecropia moth *Hyalophora cecropia*, the silkmoth, *Bombyx mori*, and the fall armyworm *Spodoptera frugiperda*, all of which develop the ovaries and eggs during some part of the pupal stage (Tsuchida et al. 1987; Sorge et al. 2000). In contrast, the noctuid moth *Heliothis virescens* (Zeng et al. 1997) and the monarch but-

terfly *Danaus plexippus* (Pan & Wyatt 1971) have a relatively immature ovary at adult emergence.

Female Phaon crescents have four ovarioles in each of 2 ovaries, and each ovariole contains about 49 egg follicles, with apparently mature eggs ready to be fertilized and laid a few days after emergence. Thus, a female might be able to lay about 400 eggs, and we found that one individual did lay 434 eggs. The failure of females produced on the host-free PB diet to lay eggs may be due to a failure to mate. Despite substantial time in observations, we never observed mating in butterflies produced on the PB diet, whereas observations of mating were common in butterflies produced on PB diet with 10% freeze-dried host leaves or those produced on living host plants. Mating is a stimulus for oviposition and oogenesis in some insects. For example, oviposition in the Australian field cricket, *Teleogryllus* sp., and the onion fly, *Delia* sp., is enhanced as a result of mating (Chapman 1998). Males of some lepidopterans transfer prostaglandins or prostaglandins-synthesizing chemicals to the female during mating and these stimulate oviposition (Stanley-Samuelson 1994).

Male Phaon crescents produced on host plants have a mature reproductive system and mate within 2-3 days after emergence. The male system includes fused testes, vas deferentia, paired accessory glands, and an ejaculatory structure and duct. The enlarged regions of the vas deferentia that serve as a sperm reservoir and seminal vesicle in males produced on the host plant or PB diet with 10% freeze-dried host plant leaves appear to be atrophied in males produced on PB diet. The testes from PB diet reared males are larger (swollen) and light red in color, compared to those reared on living host plant or PB diet with 10% freeze-dried host leaves. These defects observed in the internal reproductive system of males produced on the PB diet, coupled with the failure to get any reproduction from sexes produced on the PB diet suggest that these males may not produce viable sperm.

Although no apparent abnormalities were detected in the internal reproductive system of females produced on PB diet, they could have physiological defects in the reproductive system that are not evident from simple dissections.

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INSECTS ASSOCIATED WITH FABA BEAN, *VICIA FABA* (FABALES: FABACEAE), IN SOUTHERN FLORIDA

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ABSTRACT

One hundred faba bean (*Vicia faba* L., Fabales: Fabaceae) accessions from the USDA-NSSL Seed Repository in Prosser, WA were grown outdoors in southern Florida from December 2000 through April 2001 and October 2001 through April 2002 to both evaluate their potential as a forage crop and to initiate selections of superior genotypes. Insect herbivores and their predators were observed for feeding associations and collected for identification throughout the two seasons of trials. Sixty-one species of insect herbivores and nectaring predators and parasitoids were observed feeding on or were captured on faba bean leaves, stems, flowers, extra-floral nectaries or pods. Additionally, thirty-two species of predacious and parasitic insects were observed eating herbivorous insects or captured while searching for prey or hosts on faba beans plants. The most significant damage was caused by large populations of *Aphis craccivora* Koch (Hemiptera: Aphidae) that fed on terminals and young leaf and stem tissue. Six Coccinellidae species fed upon aphids and reproduced on the crop. Pods were damaged by reproducing populations of *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) and *Nezara viridula* (L.) (Hemiptera: Pentatomidae).

Key Words: *Aphis craccivora*, *Leptoglossus phyllopus*, *Nezara viridula*, bidens mottle mosaic, faba bean, *Vicia faba*

RESUMEN

Cien accesiones de haba (*Vicia faba* L., Fabales: Fabaceae) del Repositorio de Semillas de USDA-NSSL en Prosser, WA fueron sembradas en el campo en el sur de Florida de diciembre 2000 hasta abril 2001 y de octubre 2001 hasta abril 2002 para evaluar su potencial como cultivo de forraje y para iniciar la selección de genotipos superiores. Los insectos herbívoros y sus depredadores fueron observados para determinar las asociaciones alimentarias y recolectados para identificarlos durante dos estaciones de pruebas. Sesenta y una especies de insectos herbívoros y depredadores que se alimentaban del néctar, parasitoides que fueron observados alimentándose de la planta, o fueron capturados en las hojas, tallos, flores, néctar extra-floral o las vainas de haba. Además, treinta y dos especies de insectos depredadores y parasíticos fueron observados alimentándose de insectos herbívoros, o capturados mientras estaban buscando presas u hospederos sobre el haba. El daño más significativo fué causado por la alta población de *Aphis craccivora* Koch (Hemiptera: Aphidae) que se alimentó de los terminales y del tejido tierno de las las hojas y el tallo. Seis especies de Coccinellidae se alimentaron de los áfidos y se reprodujeron en el cultivo. Las vainas fueron dañadas por poblaciones de *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) y de *Nezara viridula* (L.) (Hemiptera: Pentatomidae) reproduciéndose sobre el cultivo.

The faba bean, *Vicia faba* L., is a cold hardy, grain legume originally domesticated in the Hindustani region of central Asia, but now cultivated from tropic to sub-arctic climates (Zeven & Zhukovsky 1975). This taxa has been artificially divided by seed size into three subspecies (Polhill & van der Maesen 1985). The broad bean (*V. faba* var. *major* Harz) is mostly grown as a grain vegetable because of its large seed size, while the horse bean (*V. faba* var. *equina* Pers.) and the pigeon or tick bean (*V. faba* var. *minor* Beck) are grown primarily for animal feed or as a green manure crop. In Europe, these two later species are referred to as "field beans" (Bond et al. 1985). In Florida, faba bean production is uncommon, and broad beans are only rarely seen in Florida gardens (Stephens 1994). However, Florida does have significant and diverse legume based indus-

tries throughout the state, which range from exotic oriental vegetables such as the winged bean (*Psophocarpus tetragonolubus* (L.) DC.) to forage legumes including clovers. Large commercial industries are in place for peanuts (*Arachis hypogaea* L.) and fresh market beans (*Phaseolus vulgaris* L.), with smaller production of cowpea (*Vigna unguiculata* (L.) Walp.) (Florida Agricultural Statistics Service 2001). Additionally, uses of feral legumes such as *Aeschynomene* spp. vacillate from weed to cover crop to domesticated forage. With the rare exception of the Austrian pea (*Pisum sativum* var. *arvense* (L.) Poiret) used by recreational hunters for deer browse, most legumes grown in Florida are warm season crops and frost intolerant. The faba bean is one of a few freeze tolerant winter legumes that could be integrated into Florida agriculture as either a vegetable or forage crop. It could enlarge the array of winter vegetable crops or be inserted into a silage cropping system that includes corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moench) to support the cattle and dairy industries. It has the ancillary benefits of nitrogen fixation and thus a reasonably low fertility requirement.

Any assessment of a crop's potential in a new region would be aided by the knowledge of the insect fauna that would be associated with its production. Insect and nematode pests of faba beans were broadly reviewed by Bardner (1983) and Cammel & Way (1983). Economically important faba bean insect pests include aphids that cause direct feeding damage and transmit plant viruses (e.g., *Aphis fabae* Scopoli, *A. craccivora* Koch, *Acyrthosiphon pisum* (Harris), and *Megoura viciae* Buckton) (Hemiptera: Aphidae), as well as leafhoppers, thrips, moth larvae, leafmining fly larvae, seed beetles and weevils. Many insect species are found on warm season legumes in Florida, some of which are considered to be commercial pests (Pernezny et al. 2004). It is reasonable to assume that some of these insects would overlap onto faba beans, but an actively growing legume crop in the winter season could host additional insect species not typically found on warm season legumes. The purpose of this research was to identify insects and their association (i.e., herbivorous, predacious, parasitic) with experimental plots of faba beans grown from October to April in southern Florida. Our findings are discussed in relation to other known insect pests of faba beans in the western hemisphere and of Florida legumes in general.

Materials and Methods

One hundred faba bean accessions in the range from PI 301011 through PI 577748 were acquired from the USDA-NSSL Seed Repository in Prosser, WA. The accessions were split planted in two seasons at the Everglades Research and Education Center, Belle Glade, Palm Beach County, Florida. Sixty-seven of these accessions were planted on December 1, 2000 and grown through April 30, 2001. Selections were made based on horticultural and agronomic characters and planted with the remaining 33 accessions in October 2001 and grown through April 2002. Plants were grown outdoors in 40 above-ground, concrete-walled production bins, 76.2 cm deep and 2.1 m long, filled with Palm Beach soil mix (50% compost, 25% clean sand, 25% bark, Odum's, West Palm Beach, FL). Seeds were planted 10 to 15 cm apart in rows spaced 46 cm on center, five rows per bin. Six seeds of each accession were planted in a row with final plant density averaging four plants per row and 20 plants per bin. Plots were provided with a complete fertilizer plus micro nutrients mixed with the soil at planting. Additional fertilizer (20-20-20 plus micro nutrients and ammonium nitrate) was applied at label rates on a regular basis from early February to early April in both seasons. The plants were grown insecticide free until March of both years when imidacloprid (Provado 1.6 Flowable, Bayer CropScience LP, Research Triangle Park, NC) was applied at 3 fl. oz per acre to control excessive populations of cowpea aphids, *Aphis craccivora*. A composite population of PI lines from seeds left over from selections from the previous season was mixed together and planted in the field on 31 October 2001 for observation and collection of associated insects. Hand-held planters (Almaco, Nevada, IA) were used to plant the seeds 10 cm apart in 4 rows 76 cm on center and 114 m long in a Lauderhill organic soil (i.e., euic, hyperthermic Lithic Medisaprists) at the Everglades Research and Education Center, Belle Glade, FL.

Plants were examined weekly for presence of insects at various times from early morning to early evening to survey the entire photophase. Observations of feeding associations with faba bean leaves, stems, flowers, and pods, as well as predacious and parasitic activity against insect herbivores was recorded whenever possible before specimens were collected and preserved for identification. Insects were identified to species where possible through the use of published systematic keys and direct comparisons with museum specimens housed at the Division of Plant Industry in Gainesville, Florida.

Results and Discussion

Plant and Nectar Feeders

Insects found in association with faba beans during the two seasons are divided into plant and nectar feeders (Table 1) and predators and parasitoids (Table 2). Notes on feeding associations are included for only those directly observed. Insects that caused visible damage to terminals,

TABLE 1. INSECTS FOUND FEEDING ON LEAVES, STEMS, FLOWERS AND PODS OF FABA BEANS AT BELLE GLADE, FLORIDA IN 2001 AND 2002.

Order	Family	Insect	Life stage[1]	Plant part
Orthoptera	Acrididae	*Chortophaga australion* Rehn & Hebard	A	Leaf
	Tettigoniidae	*Microcentrum rhombifolium* (Saussure)	A	Leaf
Thysanoptera	Thripidae	*Frankliniella bispinosa* (Morgan)	L & A	Flower
		Frankliniella insularis (Franklin)	A	Flower
		Frankliniella kelliae (Sakimura)	A	Flower
Hemiptera	Miridae	*Creontiades rubinervis* (Stal)	A	Leaf
	Lygaeidae	*Oncopeltus cayensis* Torre-Bueno	A	Stem /pod
		Oncopeltus fasciatus (Dallas)	A	
		Ozophora trinotata Barber	A	Leaf
	Pyrrhicoridae	*Dysdercus mimulus* Hussey	A	Pod
	Coreidae	*Acanthocephala femorata* (F.)	A	Pod
		Anasa scorbutica (F.)	A	Pod
		Leptoglossus phyllopus (L.)	N & A	Pod
		Zicca taeniola (Dallas)	A	Pod
	Alydidae	*Stenocoris tipuloides* (DeGeer)	A	
	Pentatomidae	*Acrosternum hilare* (Say)	N & A	Pod
		Acrosternum marginatum (Palesot de Bearvois)	A	Pod
		Edessa bifida (Say)	A	Pod
		Euschistus ictericus (L.)	A	Pod
		Euschistus quatrator Raulston	A	Pod
		Nezara viridula (L.)	N & A	Pod
		Thyanta perditor (F.)	A	Pod
	Cicadellidae	*Draeculocephala mollipes* (Say)	N & A	Leaf
		Gypona sp.	N & A	Leaf
	Delphacidae	*Perkinsiella saccharicida* Kirkaldy	A	
	Aphidae	*Acyrthosiphon pisum* (Harris)	N & A	Leaf
		Aphis craccivora Koch	N & A	Leaf and stem
	Pseudococcidae	*Planococcus citri* (Risso)	A	Root and stem
Coleoptera	Scarabaeidae	*Anomala marginata* (F.)	A	Pollen/nectar
		Euphoria sepulcralis (F.)	A	Pollen/nectar
		Trigonopeltastes delta Forster	A	Pollen/nectar
	Cantharidae	*Chauliognathus marginatus* (F.)	A	Pollen/nectar
	Chrysomelidae	*Diabrotica balteata* Leconte	A	Leaf
		Diabrotica undecimpunctata howardi Barber	A	Leaf
	Curculionidae	*Diaprepes abbreviatus* (L.)	A	Leaf
Lepidoptera	Pyralidae	*Hellula rogatalis* (Hulst)	A	
		Herpetogramma phaeopteralis (Guenee)	A	
		Spoladea recurvalis (F.)	A	
	Arctiidae	*Spilosoma virginica* (F.)	L	Leaf
	Noctuidae	*Feltia subterranea* (F.)	L	Seedling stem
		Spodoptera eridania (Cramer)	L	Leaf
	Saturniidae	*Automeris io io* (F.)	L	Leaf
	Hesperiidae	*Lerema accius* (J. E. Smith)	A	Flower
Diptera	Stratiomyidae	*Hedriodiscus trivittatus* (Say)	A	
		Hermetia illucens (L.)	A	
	Otitidae	*Euxesta annonae* (F.)	A	
	Tephritidae	*Xanthaciura insecta* (Loew)	A	
	Agromyzidae	*Liriomyza trifolii* (Burgess)	L & A	Leaf
Hymenoptera	Chrysididae	*Chrysis* sp.	A	Nectar
	Halictidae	*Agapostemon splendens* (Lepeletier)	A	Nectar
		Halictus sp.	A	Nectar
	Anthophoridae	*Xylocopa micans* Lepeletier	A	Nectar
	Apidae	*Apis mellifera* L.	A	Pollen/nectar

[1]Life stage: L, larva; N, nymph; A, adult.

TABLE 2. INSECTS FOUND FEEDING ON OR SEARCHING FOR INSECT HERBIVORES OF FABA BEANS AT BELLE GLADE, FLORIDA IN 2001 AND 2002.

Order	Family	Insect	Life stage[1]	Observed association
Predators				
Dermaptera	Forficulidae	*Doru taeniatum* (Dohrn)	A	General predator
Hemiptera	Reduviidae	*Repipta taurus* (F.)	N & A	General predator
		Sinea sp.	A	General predator
		Zelus longipes (L.)	N & A	General predator
	Pentatomidae	*Podisus maculiventris* (Say)	A	General predator
Coleoptera	Carabidae	*Calleida decora* (F.)	A	General predator
	Coccinellidae	*Brachiacantha decora* Casey	L & A	Aphid predator
		Coelophora inaequalis (F.)	L & A	Aphid predator
		Cycloneda sanguinea (L.)	L & A	Aphid predator
		Harmonia axyridis (Pallas)	L & A	Aphid predator
		Hippodamia convergens Guerin-Meneville	L & A	Aphid predator
		Olla v. nigrum (Mulsant)	L & A	Aphid predator
Diptera	Dolichopodidae	*Condylostylus* sp.	A	
		Plagioneurus univittatus Loew	A	
	Syrphidae	*Allograpta oblique* (Say)	A	
		Palpada vinetorum (F.)	A	
		Toxomerus sp.	A	
	Calliphoridae	*Phaenicia caeruleiviridis* (Macquart)	A	
	Sarcophagidae	*Sarcodexia* sp.	A	
	Tachinidae	*Lespesia* sp. 1	A	
		Lespesia sp. 2		
		Nilea sp.	A	
		Winthemia sp.	A	
Hymenoptera	Mutillidae	*Timulla* sp.	A	
	Vespidae	*Eumenes fraternus* Say	A	Lepidoptera predator/ nectar
		Pachyodynerus nasidens (Latreille)	A	Lepidoptera predator/ nectar
		Polistes dorsalis (F.)	A	Lepidoptera predator/ nectar
		Polistes major Beauvois	A	Lepidoptera predator/ nectar
		Polistes metricus Say	A	Lepidoptera predator/ nectar
	Pompilidae	*Anoplius* sp.	A	
	Sphecidae	*Liris* sp.	A	Lepidoptera predator/ nectar
Parasitoids				
Hymenoptera	Braconidae	*Bracon* sp.	A	
		Cotesia sp.	A	
	Ichneumonidae	*Coccygomimus marginellus* (Brulle)	A	
		Exetastes sp.	A	
		Pterocormus sp.	A	
		Trogomorpha trogiformis (Cresson)	A	
	Chalcididae	*Brachymeria* sp.	A	Extra floral nectary
		Conura sp.	A	Extra floral nectary

[1]Life stage: L, larva; N, nymph; A, adult.

leaves and pods appeared to be evenly distributed across the tested accessions and none were observed to be more attractive than another to the insect herbivores and natural enemies. Collection records in Tables 1 and 2 are pooled across all accessions and both study years. Sixty-one species of insect herbivores and nectaring predators and parasitoids were observed feeding or captured on faba bean leaves, stems, flowers, extra-floral nectaries or pods.

Cowpea aphids were the most abundant insects feeding on faba bean leaves in both years of the study. Their feeding was concentrated on the youngest leaf and stem tissue and resulted in stunted terminal growth and distorted leaf expansion. They are known as faba bean pests throughout the Mediterranean and some subtropical and tropical areas where they cause damage from both direct feeding and virus transmission (Cammell & Way 1983). The pea aphid,*Acyrthosiphon pisum*, was a late season colonizer of the crop after initiation of pod set in February 2001, but not in 2002. It utilized a different microhabitat of the plants compared with *Aphis craccivora*, concentrating instead on the underside of leaves in the more protected middle region of the canopy. Lowe (1967) found that *A. pisum* first preferred faba bean stems in the growing terminal before moving to developing leaves. Pea aphids are known for causing more damage from virus transmission than from direct feeding damage (Cammell & Way 1983). *Aphis fabae* is known from Florida (Halbert & Nuessly 2001) and is an aphid pest of faba bean in Nova Scotia, Canada (Patriquin et al. 1988), but it was not observed feeding on the crop in our studies.

A crippling virus, Bidens mottle mosaic, infected the PI accessions tested during the middle of the first year causing stunted terminal growth and chlorotic, disfigured leaves and pods (Baker et al. 2001). While the disease is known from southern Florida on leafy vegetables, and both of the aphids colonizing the plants in our study are known vectors, faba beans are a new host for this virus. No difference is colonization rates of accessions were observed for either cowpea or pea aphids. Two other aphid vectors of Bidens mottle mosaic, *Myzus persicae* (Sulzer) and *Aphis spiraecola* Patch (both Hemiptera: Aphidae), are known from the area (Halbert & Nuessly 2001), but they were not found feeding on or colonizing faba beans during this study. Aphid transmitted viruses have also been reported on faba bean in Guatemala (Vasquez 1988). Plants infested with broad bean mosaic virus in Egypt serve as better hosts of *A. craccivora* allowing them to produce more progeny on infected than on non-infected plants (El-Kady & Salem 1974).

Other piercing-sucking insects observed feeding on leaves (Table 1) included the plant bug *Creontiades rubinervis* (Stal), the seed bug *Ozophora trinotata* Barber, and the leafhoppers *Draeculocephala mollipes* (Say) and *Gypona* sp. Other mirids, including *Lygus* sp., have been reported to produce necrotic spots on leaves that later collapse to form holes (Bardner 1983). Leafhopper feeding damage was also noted by Bardner (1983) to produce distorted growth and stunting on faba beans. While necrotic lesions were observed on leaves in our plantings, it was not confirmed whether they were the result of feeding by these heteropterous and homopterous insects. The lygaeid *Oncopeltus cayensis* Torre-Bueno was observed probing stems and pods, while *O. fasciatus* (Dallas) was not observed feeding on any of the plant structures. Both are known to specialize on various milkweeds (Slater & Baranowski 1990).

Two species of leafminers were found attacking faba bean leaves. The American serpentine leafminer, *Liriomyza trifolii* (Burgess), is a common pest of leafy vegetables throughout Florida (Spencer & Stegmaier 1973). Damage by this insect consisted of feeding and oviposition stipples and mines on leaves, but not pods. Another species of dipterous leafminer produced much wider and longer tunnels lined with a dark residue that was quite obvious without light transmission. This leafminer remains unidentified because repeated attempts to rear adults from larvae in infested leaves held in plastic cups at room temperature were unsuccessful.

Species from several orders were found chewing on faba bean foliage (Table 1). The grasshoppers *Chortophaga australion* Rehn & Hebard and *Microcentrum rhombifolium* (Saussure) ate large jagged edge sections from leaves. Granulate cutworm, *Feltia subterranea* (F.), cut off seedling faba beans at their base. Both cucumber beetle species found in southern Florida, banded cucumber beetle (*Diabrotica balteata* Leconte) and spotted cucumber beetle (*D. undecimpunctata howardi* Barber), produced irregular sized notches on the edge and holes within the youngest fully expanded leaves. These cucumber beetles have a wide adult host feeding range and *D. balteata* is a pest of leafy vegetables and sweet corn in southern Florida (Nuessly & Webb 2002a, b). A single adult Diaprepes root weevil (*Diaprepres abbreviatus* (L.)) was found feeding on the edge of a leaf. The adults of this species have been reported to feed on a variety of vegetables and weeds and the larvae are pests of many crops, including citrus and sugarcane (Simpson et al. 1996), which are grown extensively throughout central and southern Florida. Larvae of the tiger moth (*Spilosoma virginica* (F.)) and Io moth (*Automeris io io* (F.)) were the only Lepidoptera observed to complete development on the plants. Larvae of other species, including the southern armyworm (*Spodoptera eridania* (Cramer)), were collected on plants, but were likely predated by wasps, beetles, bees and assassin bugs (Table 2) before they could complete development. Adults of three species of pyralids were captured while they rested on the plants (Table 1).

Flower and nectar feeders included thrips, beetles, skippers and wasps (Table 1). The thrips *Frankliniella bispinosa* (Morgan), *F. insularis* (Franklin), and *F. kelliae* (Sakimura) fed on pollen, anthers, and other flower parts, but did not cause any noticeable problems with pollination or seed set. Adults of three scarab beetle species were

found feeding on pollen and nectar within faba bean flowers. *Anomala marginata* (F.) and *Euphoria sepulcralis* (F.) are common flower feeders, with the latter species found feeding at ear tips and armyworm feeding holes of sweet corn (*Zea mays* L.) ears (Nuessly et al. 1999). *Trigonopeltastes delta* Forster is commonly found feeding on fragrant inflorescences of many plants, including the sable or cabbage palm (*Sabal palmetto* (Walt. Lodd.)) (G.S.N., unpublished data). The soldier beetle *Chauliognathus marginatus* (F.) became very common as the seasons progressed, feeding on nectar and pollen within flowers during late afternoon and early evening. Mating pairs were frequently observed. Adults of the hesperiid *Lerema accius* (J.E. Smith) were observed feeding on faba bean flowers. Various bees (Anthophoridae, Halictidae and Apidae) were observed feeding at the flowers (Table 1). While wasps are discussed below, paper wasps (Vespidae), spider wasps (other Sphecidae), and the cuckoo wasp *Chrysis* sp. (Tables 1 and 2) were also observed flying between and feeding from numerous flowers during the day. Two Chalcidoidae species were also observed feeding from extra floral nectaries.

Pod feeders composed the largest guild of faba bean herbivores observed in the experiments (Table 1). The pyrrhocorid *Dysdercus mimulus* Hussey, four species of Coreidae and seven species of Pentatomidae fed on developing pods. *Leptoglossus phyllopus* (L.) was the most common and destructive of the Coreidae that fed and reproduced on the crop. Their nymphs were observed to feed in small groups on pods. This species feeds on a wide variety of cultivated crops, including cowpea (Baranowski & Slater 1986). Pod feeding produced raised, pitted black bumps on the pod surface and black spots on developing seeds. The other coreids found on faba beans in our studies, *Acanthocephala femorata* (F.), *Anasa scorbutica* (F.), and *Zicca taeniola* (Dallas), are more commonly found associated with native plants and have not been identified as pests of leguminous plants (Baranowski & Slater 1986). Pod damage similar to that caused by *L. phyllopus* also was produced by the most commonly encountered stink bug, *Nezara viridula* (L.). This insect also reproduced on the faba beans, although few were observed to complete development. Six other stink bug species (Table 1) were not commonly encountered and were not observed to reproduce on faba beans. Three of these six species, *Acrosternum hilare* (Say) (Simmons & Yeargan 1990), *A. marginatum* (Palesot de Bearvois) (Hallman et al. 1985), and *Thyanta perditor* (F.) (Saunders et al. 1983) are known to cause at least some damage to soybeans or other cultivated legumes.

Dipterous species in the families Stratiomyidae, Otitidae, and Tephritidae were captured while they rested on bin and field planted faba beans, but no feeding associations were noted for these flies. These fly species are commonly found on many species of agronomic crops and weeds throughout southern Florida (G.S.N., unpubl. data).

Predacious and Parasitic Insects

Twenty-seven species of predator and parasitoid insects were collected during our studies. Larvae of six coccinellid species (Table 2) fed on cowpea aphids and their adults were reared from pupae collected from stems and under leaves of test plants. Raymond et al. (2000) found that *Aphis fabae* feeding on *V. faba* attracted the coccinellid *Adalia bipunctata* L. in laboratory testing, whereas plants without aphids or ones with aphids recently removed did not attract the beetles. Larvae of all three syrphids feed on the sugarcane aphids *Melanaphis sacchari* (Zehntner) and *Sipha flava* (Forbes) (both Hemiptera: Aphidae) in Florida sugarcane fields (Hall 1988).

Calleida decora (F.) is a red and iridescent green predacious ground beetle commonly encountered on various cultivated crops throughout the southeastern and into the mid-western United States (Erwin et al. 1977). Larvae and adults of this species were found on the soil and up into the faba bean canopy. It is an important predator of several lepidopterous pests of cotton and soybean (Harris et al. 1985).

Solitary and social wasps (Sphecidae and Vespidae, Table 2) were frequently observed searching leaves that exhibited feeding damage. These wasps normally anesthetize their prey and then either macerate them into "meat balls" to bring back to their nests or use them to provision solitary mud or sub-soil nests for their progeny. Feeding damage associated with medium through large sized Lepidoptera larvae was not common on our faba beans and a few late instar southern armyworm, tiger, and io moth larvae were the only large larvae found. Resistance to armyworm pests in faba beans was not noted in the Clement et al. (1994) review of plant resistance achievements in cool season food legumes. Therefore, we believe that lepidopteran larvae in their early to mid instars succumbed to predation rather than to plant resistance mechanisms.

The assassin bugs *Repipta taurus* (F.), *Zelus longipes* (L.) and *Sinea* sp. were each observed to feed on Lepidoptera larvae, cucumber beetle adults and spiders on faba bean leaves. They are generalist predators found throughout the United States (Blatchley 1926; Reinert 1978; Altieri & Whitcomb 1980). The earwig *Doru taeniatum* (Dohrn) and velvet ant *Timulla* sp. were captured on faba beans without any specific feeding association, however, the former is known as a predator of armyworms, aphids and other soft bodied insects in corn (Jones 1985) and sugarcane (Hall 1988).

Several species of insects caused damage to the crop in our studies. Aphid (*Aphis craccivora*) feed-

ing on terminals and virus infection by colonizing and non-colonizing aphids reduced the growth and reproduction of the crop. Feeding by seed (*Leptoglossus phyllopus*) and stink bugs (*Nezara viridula*) also caused damage to seeds developing within pods. However, other species with known pest associations with warm season beans in Florida were not encountered on faba beans in these limited tests. These included the following important pests of snap beans in Florida (Pernezny et al. 2004; Capinera 2001): melon thrips (*Thrips palmi* Karney) (Thysanoptera: Thripidae), cowpea curculio (*Chalcodermus aeneus* Bohemanor) (Coleoptera: Curculionidae), bean leafroller (*Urbanus proteus* (L.)) (Lepidoptera: Hesperiidae) and lesser cornstalk borer (*Elasmopalpus lignosellus* (Zeller)) (Lepidoptera: Pyralidae).

Faba bean accessions grew well in the south Florida Winter climate during both seasons. Plants produced main stems up to 2.29 m long in the first season during which there were five freeze events, with one down to -6.1°C. These cool season plants grew main stems only 1.83 m long during the warmer second season during which there were no freeze events. Yields of accessions selected for greatest pod development and vegetative growth will be determined in larger block studies to be conducted in following seasons.

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ANTS (HYMENOPTERA: FORMICIDAE) OF BERMUDA

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ABSTRACT

For more than 50 years, two exotic ant species, *Linepithema humile* (Mayr) and *Pheidole megacephala* (F.), have been battling for ecological supremacy in Bermuda. Here we summarize known ant records from Bermuda, provide an update on the conflict between the dominant ant species, and evaluate the possible impact of the dominant species on the other ants in Bermuda. We examined ant specimens from Bermuda representing 20 species: *Brachymyrmex heeri* Forel, *B. obscurior* Forel, *Camponotus pennsylvanicus* (De Geer), *Cardiocondyla emeryi* Forel, *C. obscurior* Wheeler, *Crematogaster* sp., *Hypoponera opaciceps* (Mayr), *H. punctatissima* (Roger), *L. humile*, *Monomorium monomorium* Bolton, *Odontomachus ruginodis* Smith, *Paratrechina longicornis* (Latreille), *P. vividula* (Nylander), *P. megacephala*, *Plagiolepis alluaudi* Forel, *Solenopsis* (*Diplorhoptrum*) sp., *Tetramorium caldarium* Roger, *T. simillimum* (Smith), *Wasmannia auropunctata* (Roger), and an undetermined Dacetini. Records for all but three (*H. punctatissima*, *P. vividula*, *W. auropunctata*) include specimens from 1987 or later. We found no specimens to confirm records of several other ant species, including *Monomorium pharaonis* (L.) and *Tetramorium caespitum* (L.). Currently, *L. humile* dominates most of Bermuda, while *P. megacephala* appear to be at its lowest population levels recorded. Though inconspicuous, *B. obscurior* is common and coexists with both dominant species. *Paratrechina longicornis* has conspicuous populations in two urban areas. Three other ant species are well established, but inconspicuous due to small size (*B. heeri*, *Solenopsis* sp.) or subterranean habits (*H. opaciceps*). All other ant species appear to be rare, including at least one, *O. ruginodis*, which was apparently more common in the past.

Key Words: Atlantic islands, biodiversity, exotic ants, *Pheidole megacephala*, *Linepithema humile*, tramp ants

RESUMEN

Durante más de 50 años, dos especies exóticas de hormiga, *Linepithema humile* (Mayr) y *Pheidole megacephala* (F.), han estado combatiendo la supremacía ecológica en Bermuda. Aquí resumimos los registros conocidos de hormiga de Bermuda, ponemos al día el estado de la cuestion en el conflicto entre la especie dominante de hormiga, y evalúamos el impacto posible de la especie dominante en las otras hormigas en Bermuda. Examinamos especímenes de hormiga de Bermuda que representan 20 especies: *Brachymyrmex heeri* Forel, *B. obscurior* Forel, *Camponotus pennsylvanicus* (De Geer), *Cardiocondyla emeryi* Forel, *C. obscurior* Wheeler, *Crematogaster* sp., *Hypoponera opaciceps* (Mayr), *H. punctatissima* (Roger), *L. humile*, *Monomorium monomorium* Bolton, *Odontomachus ruginodis* Smith, *Paratrechina longicornis* (Latreille), *P. vividula* (Nylander), *P. megacephala*, *Plagiolepis alluaudi* Forel, *Solenopsis* (*Diplorhoptrum*) sp., *Tetramorium caldarium* Roger, *T. simillimum* (Smith), *Wasmannia auropunctata* (Roger), y un Dacetini indeterminado. Los registros para todo excepto tres (*H. punctatissima*, *P. vividula*, *W. auropunctata*) incluyen los especímenes de 1987 o más tarde. No encontramos ningún especímen para confirmar los registros de varias otras especies hormiga, inclusyendo *Monomorium pharaonis* (L.) y *Tetramorium caespitum* (L.). Actualmente, *L. humile* domina la mayor parte de Bermuda, mientras *P. megacephala* parece estar en su nivel de población más baja que se haya registrado. Aunque pasa inadvertido, *B. obscurior* es común y coexiste con ambas especies dominantes. *Paratrechina longicornis* tiene poblaciones visibles en dos áreas urbanas. Otras tres especies de hormiga se han establecido bien, aunque no llanan la atencion debido al tamaño pequeño (*B. heeri*, *Solenopsis* sp.) o hábitos subterráneos (*H. opaciceps*). Todas las otras especies hormigas parecen ser raras, incluyendo por lo menos una, *O. ruginodis*, que era más común en el pasado.

Translation provided by the authors.

A battle for territorial supremacy has been raging on the Atlantic islands of Bermuda for more than 50 years. The combatants are two species of exotic ants, one Old World (the big-headed ant, *Pheidole megacephala* (F.)) and one New World (the Argentine ant, *Linepithema humile* (Mayr)). As documented in studies conducted 1927-1986 (Haskins 1939; Haskins & Haskins 1965, 1988;

Crowell 1968; Lieberburg et al. 1975), *P. megacephala* was the dominant ant in Bermuda when *L. humile* arrived in the 1940s. This new invader quickly overran much territory, excluding *P. megacephala*. *Pheidole megacephala*, however, persisted, and ever since, these two species have been contesting ever-shifting battlefronts between mutually exclusive territories. Largely ignored in this drama, however, are the other ant species in Bermuda.

Both *P. megacephala* and *L. humile* are well-known for killing off native invertebrates, particularly native ants (Erickson 1971; Human & Gordon 1996; Holway 1999; Vanderwoude et al. 2000; Wetterer et al. 2000, 2001; Wetterer 2002). This paper presents combined published, unpublished, and new ant records from Bermuda, provides an update on the conflict between the two dominant ant species, and examines the possible impact of the dominant ants on the other ant species that persist in Bermuda.

Published Ant Records from Bermuda

Many early accounts describe enormous ant plagues in Bermuda in the 17th and 19th centuries (Jones 1859; LeFroy 1882; Hurdis 1897; Kevan 1981), but no specimens of these ants are known and the species involved have never been identified. Wheeler (1906) proposed that the plague ants might have been *Solenopsis geminata* (F.) or *Monomorium destructor* (Jerdon).

In the first identification of an ant species from Bermuda, Kirby (1884) of the British Museum identified one ant species collected by the HMS Challenger expedition in April 1873 as *Formica nigra* L. (= *Lasius niger* (L.)). Kirby (1884) noted that this species was "probably introduced" and that "the specimens do not appear to differ from the ordinary European species."

Dahl (1892) identified two species of ants from Bermuda, collected in 1889 by the Humboldt-Stiftung Expedition, as *Pheidole pusilla* Heer (= *P. megacephala*) and an *Odontomachus* species, "probably" *Odontomachus insularis* Guérin-Méneville.

Verrill (1902) reported that on expeditions to Bermuda in 1898-1901 "ants of several undetermined species were collected by us which have not yet been fully studied by a specialist." Nonetheless, Verrill (1902) recognized specimens of the "small House-ant" *Monomorium minutum* (Buckley) (= *Monomorium monomorium* Bolton) and the "Garden-ant or Pavement-ant" *Tetramorium caespitum* (L.), and wrote: "probably these were early introduced from England." In addition, Verrill (1902) received from V. Hayward specimens of *P. megacephala* collected on St. David's Island, and received from L. Mowbray "a few Hymenoptera, including males, females, and very small workers of one or two species of the genus *Pheidole*, as determined by Mr. Th. Pergande. These are common, as House-ants, and destructive." Verrill (1902) also mentioned "a few ants," found in guts of the endemic Bermuda lizard *Eumeces longirostris* Cope. Finally, Verrill (1902) cited Kirby's (1884) record of the "European Black ant" *L. niger*, and Dahl's (1892) records of *P. megacephala* and *Odontomachus* sp. near *insulans*.

Wheeler (1906) made a comprehensive list of Bermuda ants, based on the three past accounts (Kirby 1884; Dahl 1892; Verrill 1902) and on new specimens supplied by T. Kincaid and J. H. Comstock. Of the 11 taxa on his list, Wheeler (1906) examined specimens of eight: *Hypoponera opaciceps* (Mayr), *Odontomachus haematodes insularis ruginodis* Smith (= *O. ruginodis*), *Cardiocondyla emeryi* Forel, *P. megacephala*, *Brachymyrmex heeri* Forel, *Brachymyrmex heeri obscurior* Forel (= *B. obscurior*), *Prenolepis kincaidi* Wheeler (= *Paratrechina vividula* (Nylander)), and *Prenolepis* sp. The other three records came from previously published reports: *L. niger*, *M. minutum* (= *M. monomorium*), and *T. caespitum*. Wheeler (1906) believed, however, that the last two records were probably misidentifications of *Monomorium pharaonis* (L.) and *Tetramorium guineense* (Bernard) respectively.

Ogilvie (1928) presented a Bermuda ant list that was the same as Wheeler's (1906), except that it included *O. haematodes insularis* (= *O. insularis*) instead of *O. ruginodis*, and omitted *Prenolepis* sp.

Starting in 1927, Haskins repeatedly visited Bermuda, recording the ecological dominance of *P. megacephala* (Haskins 1939). Haskins (1939) noted that *O. insularis* was common in 1927, but became rare in the 1930s, writing: "In the few *Odontomachus* colonies remaining on the Islands great numbers of *Pheidole* workers are to be found killing and carrying off the larvae, fastening themselves in myriads to the bodies of the workers, and forcing their early abandonment of the site. Within another ten years, the Ponerine [*Odontomachus*] species, which inhabited Bermuda as its undisturbed Arthropod mistress for millennia, and has in fact developed a characteristic variety there, will have been exterminated."

The first published record of *L. humile* in Bermuda included it as prey recovered from stomachs of exotic *Anolis* lizards (Simmonds 1958). Simmonds (1958) found 4105 prey in 176 *Anolis grahami* Gray specimens, of which 2176 were ants (21% *L. humile*, 26% *P. megacephala*, 52% *Brachymyrmex* sp., 2% ant species A), and 587 prey in 46 *Anolis leachi* Duméril & Birron specimens of which 153 were ants (97% *L. humile*, 3% *Brachymyrmex* sp.). Bennett & Hughes (1959) reported that *L. humile* "was first recorded in Bermuda in 1948 and has since become numerous." Further, Bennett & Hughes (1959) reported that *L. humile* was gradually replacing *P. megacephala*. Nonetheless, Wingate (1965) found that *P. megacephala* was still common among 319 ant prey of 30 *Anolis roquet* (Lacépède) (12% *L. humile*, 85% *P. megacephala*, 3% *B. obscurior*).

Haskins & Haskins (1965) documented interactions in Bermuda between *P. megacephala* and *L. humile*, with some mention of other ant species, e.g., noting that "in 1933, no *O. insularis* could be found," and that areas not occupied by either of the dominant species, "were extensively occupied by colonies of *B. heeri*, and required considerably more careful examination. Occasional colonies of *Ponera opaciceps* [= *H. opaciceps*] were also found in such areas."

Crowell (1968) further studied *P. megacephala* and *L. humile* in Bermuda and noted four other ant species: *B. obscurior*, *O. insularis*, *Wasmannia auropunctata* (Roger), and *Paratrechina* sp. Crowell (1968) wrote: "the presence of *Wasmannia auropunctata* has been recognized by the Bermuda Department of Agriculture and Fisheries since 1950." Lieberburg et al. (1975), in another study of *L. humile* and *P. megacephala* in Bermuda, noted six other ant species: *B. heeri*, *H. opaciceps*, *Odontomachus brunneus* (Patten), *W. auropunctata*, *Cardiocondyla* sp., and *Paratrechina* sp. Crowell (1968) added a personal communication from C. Haskins who found one *O. insularis* colony in 1965.

Kempf (1972), in his catalog of Neotropical ants, listed ten taxa known from Bermuda: *B. heeri*, *Brachymyrmex heeri aphidicola* Forel (= *B. obscurior*), *B. obscurior*, *C. emeryi*, *H. opaciceps*, *O. insularis*, *O. ruginodis*, *P. megacephala*, *Plagiolepis alluaudi* Forel, and *T. caespitum*. Brandão (1991), in his addendum to Kempf's (1972) catalog, listed *B. obscurior*, *O. brunneus*, *O. insularis*, and *P. vividula* from Bermuda.

Haskins & Haskins (1988) revisited Bermuda for a "final survey" of *P. megacephala* and *L. humile*. Haskins & Haskins (1988) wrote that "the genus *Odontomachus* (*insularis* and *brunnei*) . . . is now a rare form. Other long-term survivors include the genus *Brachymyrmex* (still relatively abundant in niches unoccupied by either tramp ant) and the genera *Paratrechina*, *Cardiocondyla*, *Hypoponera*, and *Wasmannia*."

Hilburn et al. (1990) listed 14 ant taxa reported from Bermuda, eight apparently based on specimens (*B. heeri*, *Brachymyrmex* sp., *C. emeryi*, *L. humile*, *Monomorium* sp., *Paratrechina* sp., *P. megacephala*, and *W. auropunctata*) and six apparently from published reports (*H. opaciceps*, *L. niger*, *M. pharaonis*, *O. brunneus*, *P. vividula*, and *T. caespitum*). Hilburn et al. (1990) also listed three additional ant species that had been intercepted on goods being imported into Bermuda, but had not become established (*Camponotus noveboracensis* (Fitch), *Crematogaster* sp., and *Paratrechina longicornis* (Latreille)).

Materials and Methods

We looked for Bermuda ant specimens in the collections of the American Museum of Natural History, New York (AMNH), the Academy of Natural Sciences, Philadelphia (ANS), the Bermuda Aquarium, Museum and Zoo (BAMZ), the Bermuda Dept. of Agriculture (BDOA), British Natural History Museum in London (BNHM), Harvard's Museum of Comparative Zoology (MCZ), the Smithsonian Institute (SI), and Yale's Peabody Museum (YPM).

From 27 February to 5 March 2002, we surveyed ants using visual search in a wide range of habitats. Our sites included both highly disturbed environments (e.g., port areas in Hamilton, St. George, and Ireland Island North) and lesser-disturbed reserve areas (e.g., Spittal Pond and Paget Marsh). We also surveyed ants on two small, isolated islands, Nonsuch and Horn. These two islands are nesting areas for the endemic cahow (*Pterodroma cahow* Nichols & Mowbray). In addition, we resurveyed ten sites that Haskins & Haskins (1988) had repeatedly surveyed to evaluate changes over time in which ant species dominated an area. In June-August 2002, A. Lines, W. Sterrer, and Z. Amaral of the BAMZ collected additional ant specimens.

Stefan Cover examined most specimens. Mark Deyrup examined all specimens with uncertain identifications. Further evaluations were made by Xavier Espadaler (*Monomorium*, *Plagiolepis*), Bernhardt Seifert (*Cardiocondyla*), and James Trager (*Paratrechina*, *Crematogaster*). We will deposit vouchers at the BAMZ, MCZ, and Archbold Biological Station.

Results

We examined ant specimens from Bermuda representing 20 ant species, including nine new records (Table 1; for details species accounts). At the BNHM, we did not find the specimens Kirby (1884) identified as *Lasius niger*. At the YPM, Raymond Pupedis (pers. comm.) found catalog numbers for Hymenoptera specimens in alcohol collected in Bermuda: 4916-4928 (April 1901, AE Verrill & WJ Van Name) and 5003-5008 (Dec 1901, TG Goslin). We did not, however, find any of Verrill's ant specimens in the pinned collection. Chris Cutler searched through all available old vials and bottles in the Yale collection with no success.

In 2002, we found *L. humile* in large numbers at all ten sites studied by Haskins & Haskins (1988; see Table 2). At four of the sites, we also found *P. megacephala* (Table 2). At the intersection of Knapton Hill Road and Harrington Hundreds, we found *L. humile* to the north of Knapton Hill Road and *P. megacephala* south of the road. At Spittal Pond Reserve, we found *L. humile* throughout, except for *P. megacephala* at the eastern entrance and parking area. At Newstead Hotel complex, we found *L. humile* throughout, except for *P. megacephala* at the westernmost end. Finally, on Ireland Island North, we found *L. humile* in all areas we searched, except for

TABLE 1. ANTS OF BERMUDA.

	2000-2002 records	Record dates	Range	Status
Linepithema humile	27	1948-2002	T–F–XAME	NX
Brachymyrmex obscurior	19	1905-2002	TWFBX–––	N?
Pheidole megacephala	17	1889-2002	TWFBXAME	OX
+*Paratrechina longicornis*	7	1990-2002	TWFBXAME	OX
Brachymyrmex heeri	6	1905-2002	TWFBX––E	N?
Hypoponera opaciceps	4	1905-2002	TWFBX–––	N?
+*Solenopsis* sp.	4	1934-2002	X	??
+*Tetramorium simillimum*	2	1922-2002	TWFBX––E	OX
+*Camponotus pennsylvanicus*	2	2001-2002	––F–X–––	NX
Odontomachus ruginodis	1	1889-2002	TWFBX–––	N?
Monomorium monomorium	1	1900-2002	–W––X––E	OX
Cardiocondyla emeryi	1	1905-2002	TWFBX–M–	OX
+*Tetramorium caldarium*	1	2002	TWFBXAME	OX
Plagiolepis alluaudi		1945-1987	–W––X–––	OX
+*Cardiocondyla obscurior*		1987	–WF–X–––	OX
+*Crematogaster* sp. male		1987	X	??
+Dacetine male		1987	X	??
Wasmannia auropunctata		1925-1966	TWFBX––E	NX
Paratrechina vividula		1905-1925	TWFBX––E	OX
+*Hypoponera punctatissima*		1910	TWFBXAME	OX
Unconfirmed records				
Paratrechina sp.		1966-1973	?	??
Prenolepis sp.		1905	?	??
Tetramorium caespitum		1900	T–––?A–E	O?
Lasius niger		1873	––––?––E	O?
Monomorium pharaonis		?	TWFB?–ME	OX

Species ranked according to number of collection sites in 2000-2002 or date last recorded. + = new record for Bermuda. Range: T = Tropical South and Central America, W = West Indies, F = Florida, B = Bahamas, X = Bermuda, A = Azores, M = Madeira, E = Europe. Status: N = New World native, O = Old World native, X = exotic, ? = possible native.

P. megacephala on the northeast corner, east of the entrance to the Maritime Museum and out the entire length of the North Breakwater, which serves as a cruise ship terminal.

Species Accounts

+ = new record for Bermuda. Collectors: H = DJ Hilburn et al., W = JK Wetterer & AL Wetterer in 2002, Collections: BDOA = Bermuda Department of Agriculture, BAMZ = Bermuda Museum, Aquarium and Zoo, BNHM = British Natural History Museum, London, MCZ = Museum of Comparative Zoology, Harvard University, YPM = Yale Peabody Museum.

1. *Brachymyrmex heeri* Forel

Specimens examined: No site data (1905, T Kincaid, MCZ). Near Hamilton (1910, EG Vanatta, ANS). Padet (sic.) Marsh (1922, HH Whelzel, MCZ). Paget (1925, L Ogilvie, MCZ). No site data (1925, L Ogilvie, AMNH). Hamilton (1934, NA Weber, MCZ). Many sites (1987-1988, H, BAMZ). Admiralty House (1987, H, BAMZ - male labeled "prob. Dolichoderine male det DR Smith"). BAMZ Ops (2001, L. Hinton, BAMZ). Bermuda Biological Station for Research (BBSR), under boards in wooded area (W). Blue Hole Park, forested area (W). Hamilton, waterfront, in a flower planter (W). Wreck Road (W). Jenning's Road (2002, A Lines).

Wheeler (1906), Haskins (1939), Haskins & Haskins (1965), Kempf (1972), Lieberburg et al. (1975), and Hilburn et al. (1990) all recorded this species in Bermuda and it was the most common ant in the collections of Kincaid (in 1905) and Ogilvie (in 1925) in the MCZ. It was also common in Hilburn et al.'s collection of 1987-88. We collected this species in both natural and highly disturbed areas. This very small, orange, New World species is widespread and probably fairly common in Bermuda, but often overlooked because of its very small size.

2. *Brachymyrmex obscurior* Forel

Specimens examined: No site data (1905, T Kincaid, MCZ). Paget (1925, L Ogilvie, MCZ). Hamilton (1934, NA Weber, MCZ). Hamilton (1966, KM, BAMZ, male and queen). Paget (1971,

TABLE 2. SITES SURVEYED BY HASKINS & HASKINS (IN 1963-1986) AND THE PRESENT STUDY (IN 2002).

	Year				
Site	1963	1966	1973	1986	2002
Great Head Park	—	—	both	L	L
Mullet Bay Rd. & Ferry Road	P	both	both	L	L
Leamington Caves	L	—	P	L	L
Knapton Hill Intersection	L	—	both	L	L
Knapton Hill/Harrington 100s	L	—	P	L	both
Christchurch/Brighton Hill	both	—	P	L	L
Spittal Pond	P	—	P	P	both
Newstead Hotel	L	—	L	both	both
Wreck Road	both	—	both	P	L
Ireland Island	—	P	—	P	both
P. megacephala/*L. humile* sites	0.7	2.0	1.6	0.6	0.4

P = *Pheidole megacephala*, L = *Linepithema humile*, both = both species, — = not sampled.

N Krauss, SI). Many sites (1987-1988, H, BAMZ). Paget Parish (1987, H, BAMZ, labeled "*Paratrechina* sp. det DR Smith"). St. George's (1987, R Gordon, BAMZ, queen, labeled "*Paratrechina* sp. det. D.R. Smith"). 19 sites (W).

Wheeler (1906), Simmonds (1958), Wingate (1965), Crowell (1968), Kempf (1972), Haskins & Haskins (1988), and Brandão (1991) all recorded the presence of *B. obscurior* in Bermuda. As noted in the specimens listed above, we found *B. obscurior* specimens in the BAMZ collection misidentified as "*Paratrechina* sp." Many others were labeled "*Brachymyrmex* sp.". Records listed as *Brachymyrmex* sp. and *Paratrechina* sp. by Hilburn et al. (1990) were probably all *B. obscurior.*

We collected *B. obscurior* at 19 locales across Bermuda, often in areas with dense populations of *P. megacephala* or *L. humile*. In some localities, where neither *P. megacephala* or *L. humile* were present (e.g., forest areas near Blue Hole), we found only this species and/or *B. heeri*. It appears to be the second most common ant species in Bermuda, after *L. humile*. We expect that a close inspection would find these ants at virtually every site in Bermuda. This New World species is extremely variable in size and color, making identification much more difficult.

+3. *Camponotus pennsylvanicus* (De Geer)

Specimens examined: Rockville Close, inside house (2002, E Beek, BDOA). Same site (W). Rockville Close, Bermuda Lumber Company (W).

A resident in Rockville Close reported to the BDOA that ants first she exterminated in her house in August 2001 had returned in January 2002. We collected specimens at the same house. At a lumberyard a few blocks away, employees told us that they often saw large ants. We searched an area where they had killed the ants earlier that day under Virginia cedar lumber from Florida, and found one live and several dead *C. pennsylvanicus* workers. It is unclear whether this North American carpenter ant is actually established in Bermuda. This species has a broad range in the US, from Pennsylvania to Florida, so it seems likely that climate would not limit its establishment in Bermuda. The BDOA had a number of samples of this species intercepted by quarantine in the past few years, often on imported Christmas trees and lumber (see below).

4. *Cardiocondyla emeryi* Forel

Specimens examined: No site data (1905, T Kincaid, AMNH). Ireland Island North, in grassy area outside clayworks (W).

Wheeler (1906), Kempf (1972), Lieberburg et al. (1975), Haskins & Haskins (1988), and Hilburn et al. (1990) also noted the presence of *C. emeryi*. This African native, though apparently not very common, certainly appears to be established in Bermuda. Due to its very small size, it is probably often overlooked.

+5. *Cardiocondyla obscurior* Wheeler

Specimen examined: Paget Parish (1987, H, BAMZ). Identified by S. Cover & B. Seifert.

This Old World tramp species is often misidentified as another tramp, *C. wroughtonii*, but may be distinguished from this species based on coloration and discriminate function analysis (Seifert 2003). Due to its small size, the species often may be overlooked.

+6. *Crematogaster* sp. male

Specimen examined: Berry Hill Road, light trap (1987, H, BAMZ, one male). James Trager identified this specimen as *Crematogaster* sp., Mark Deyrup concurred.

This species, collected only once, appears to be rare in Bermuda.

7. *Hypoponera opaciceps* (Mayr)

Specimens examined: Spittal Pond, black light (1987, H, BAMZ, one queen). Spittal Pond, under rock (W). Spittal Pond, near Spanish Rock (W). Ireland Island North, under board by dock (W, one queen), Jenning's Road (2002, A Lines).

Wheeler (1906), Haskins & Haskins (1965, 1988), Kempf (1972), and Lieberburg et al. (1975) reported this species. Hilburn et al. (1990) questioned this record as "probably misidentified or no longer established," but the BAMZ actually had a specimen collected by Hilburn. We found a small area near Spanish Rock where *H. opaciceps* was the only species present. This New World native is largely subterranean, and often overlooked.

+8. *Hypoponera punctatissima* (Roger)

Specimen examined: Hamilton (1910, EG Vanatta, ANS, one queen).

We have seen only one specimen of *H. punctatissima* from Bermuda. It is a well-known tramp species distributed throughout the tropics and subtropics and almost certainly an exotic in Bermuda. Due to its subterranean habits, *H. punctatissima* is probably often overlooked.

9. *Linepithema humile* (Mayr)

Specimens examined: No site data (1953, FD Bennett, BNHM). Many sites (1987-1988, H, BAMZ). 25 sites in Bermuda (W). Walsingham Jungle (2002, A. Lines). Spittal Pond (2002, A. Lines).

Starting with Bennett & Hughes (1959), every paper on Bermuda ants recorded this species. This South American native is currently the most common ant in Bermuda in both terms of the number of sites we found this species and in terms of its extremely high densities at these sites. We found this ant almost everywhere we collected in Bermuda, though we did not find it on three small islands we surveyed: Nonsuch Island, Horn Island, and Ordinance Island.

10. *Monomorium monomorium* Bolton

Specimens examined: Spittal Pond (1987, H, BAMZ, male labeled "*Monomorium* sp. male det DR Smith"). Ordinance Island, flowerbeds (W). Identified by X. Espadaler.

Verrill (1902) identified *Monomorium minutum* (= *M. monomorium*) and specimens of *M. monomorium* collected in 1987 and 2002 support this identification. Hilburn et al. (1990) list the above 1987 specimen as *Monomorium* sp. We collected this species at only one site, Ordinance Island, a small island where ships dock, connected to the town of St. George's by a bridge. *Monomorium monomorium* is common in the Mediterranean. In the West Indies it has been recorded in Barbados (Kempf 1972).

11. *Odontomachus ruginodis* Smith

Specimens examined: No site data (1905, T Kincaid, AMNH). Near Sharks Hole (1910, EG Vanatta, ANS, labeled *O. haematodes insularis* det Gregg 1956). Nonsuch Island (1931, no collector data, AMNH, labeled *O. haematodes insularis*). Walsingham Jungle (2002, A Lines & W Sterrer, BAMZ). Identified by M. Deyrup.

Dahl (1892) identified Bermuda specimens as "probably" *Odontomachus insularis*. Wheeler (1906) recorded a closely related variety, now considered a separate species: *O. ruginodis*. Later authors list one or two *Odontomachus* species from Bermuda: *O. insularis* and/or *O. brunneus*. Based on worker morphology, Brown (1976) regarded *O. ruginodis* as synonymous with *O. brunneus*. Brown (in Deyrup et al. 1985), however, changed his mind, and again separated them into two distinct species. Because all specimens that we examined were *O. ruginodis*, we will assume that all other published records were this species as well.

Jeremy Madeiros (Bermuda Department of Conservation Services, pers. comm.) reported seeing this large trap-jaw ant twice at night in 2001, on Long Rock and near Spanish Rock. We searched the area around Spanish Rock for more than an hour without finding this ant. After we left Bermuda, Alex Lines & Wolfgang Sterrer of the BAMZ, collected two specimens in Walsingham. This species used to be common in Bermuda but now appears to be quite rare (see Introduction). It is considered to be native to the West Indies and the Bahamas (Deyrup et al. 1998), but may be exotic in Florida (Deyrup 1991).

+12. *Paratrechina longicornis* (Latreille)

Specimens examined: Brighton Nursery, on *Poinsettia* from California (1990, no collector data, BDOA). Hamilton, three sites (W). Ireland Island North, four sites (W).

Hilburn et al. (1990) recorded this species as intercepted on imported plants in 1971 and on imported *Dahlia* bulbs in 1987, but considered it not established in Bermuda. We found *P. longicornis* well established over broad stretches of the Hamilton waterfront as well as on a large portion of Ireland Island North. This conspicuous Old World tramp has never before been recorded out of quarantine in Bermuda.

13. *Paratrechina vividula* (Nylander)

Specimens examined: No site data (1905, T Kincaid, MCZ, types for *Prenolepis kincaidi*). Paget (1925, L Ogilvie, MCZ).

Paratrechina vividula has not been collected since 1925 and may be extinct in Bermuda. This Old World tramp species has been widely distributed through human commerce.

14. *Pheidole megacephala* (F.)

Specimens examined: No site data (no date, Pergande collection, SI, "480" - probably collected ~1890). No site data (1905, T Kincaid, MCZ & AMNH), Five sites (1910, EG Vanatta, ANS). Padet Marsh (1922, HH Whelzel, MCZ). Cooper's Island (1922, HC Hoyt, ANS). Hamilton (1934, NA Weber, MCZ). Paget (NLH Krauss, 1971, SI). Many sites (1987-1988, H, BAMZ). 15 sites (W). Brimstone Hill (2002, Z. Amaral). Lambda Island (2002, A. Lines).

Every paper on Bermuda ants beginning with Dahl (1892) has recorded *P. megacephala*. Of 122 specimens collected by Vanatta in 1910, 117 were *P. megacephala*, suggesting that this species was dominant in Bermuda at this time. At the ten sites repeatedly surveyed since 1963, the latest survey found *P. megacephala* at four sites and *L. humile* at all ten, the lowest ratio of *P. megacephala* to *L. humile* yet recorded. Still, we found *P. megacephala* in numerous other sites in Bermuda, including three islands where *L. humile* was absent: Nonsuch Island, Horn Island, and Ordinance Island.

15. *Plagiolepis alluaudi* Forel

Specimens examined: No site data (1945, Stern & Pruit, SI, "NY-95303 46-1072 Surinam Cherry lvs"). No site data (1950, no collector data, SI, "NY110550 50-3046 on *Zebrina pendula* cut"). No site data (1950, no collector data, SI, "52-3330"), Warwick Parish (1987, J Hendrickson, BAMZ, "Brightside on *Cassia*").

Kempf (1972) listed this species in the New World from Bermuda, St. Kitts, and St. Lucia. This small orange ant is an African tramp species that has been spread around the world, particularly in the Pacific, through human commerce (Wilson & Taylor 1967).

+16. *Solenopsis* (*Diplorhoptrum*) sp.

Specimens examined: Hamilton (1934, NA Weber, MCZ). Brimstone Hill (2000, no collector data, BDOA), BBSR, under boards and under concrete (W). Hamilton, waterfront, in planters (W). Hamilton, around Ambouy Point (W).

We suspect that this small orange thief ant is probably common throughout Bermuda, but generally overlooked due to its size and primarily subterranean habits. Thief ants commonly persist at high densities in areas invaded by dominant exotic ants such as *P. megacephala* and *L. humile* (Wetterer et al. 2001). The taxonomy of thief ants is in disarray and more than one species of thief ant may have been collected in Bermuda.

+17. *Tetramorium caldarium* Roger

Specimen examined: Newstead Hotel complex, west end (W).

We collected a single *T. caldarium* worker found battling with a *P. megacephala* worker on a bare dirt bank. It is the only species that we found for the first time in 2002. This Old World tramp species appears to be rare in Bermuda.

+18. *Tetramorium simillimum* (Smith)

Specimens examined: Padet Marsh (1922, HH Whelzel, MCZ). Ferry Point Park entrance, side of the road (W). Devonshire, Happy Talk Road (2002, A. Lines).

This Old World tramp species seems to have a long history in Bermuda but remains rare.

19. *Wasmannia auropunctata* (Roger)

Specimens examined: Paget (1925, L Ogilvie, MCZ).

We examined one *W. auropunctata* specimen collected by Ogilvie, though this species was not on Ogilvie's (1928) list. Crowell (1968) recorded *W. auropunctata* and mentioned a 1950 record. Lieberburg et al. (1975), Haskins & Haskins (1988), and Hilburn et al. (1990) also reported *W. auropunctata*. Hilburn et al. (1990) wrote that this species is "now fairly common." However, because we did not collect this ant and did not find any specimens collected by Hilburn, we believe that Hilburn and others may have mistaken other small orange ants in Bermuda (e.g., *B. heeri*, *P. alluaudi*, or *Solenopsis* sp.), as being *W. auropunctata*. Populations of *W. auropunctata* may have declined or become extinct in Bermuda. This ant was first recorded in Florida in 1924 and soon became a major pest. However, densities of *W. auropunctata* appear to have declined in many parts of Florida (Deyrup et al. 2000). Bermuda is the northernmost outdoor locale recorded for *W. auropunctata* (Wetterer & Porter 2003).

+20. Dacetine male

Specimen examined: Paget Parish, Malaise trap (1987, H, BAMZ, one male, labeled "Myrmacinae male, det DR Smith").

No dacetines have been previously reported from Bermuda. Unfortunately, no one could identify this specimen to genus. Barry Bolton (BNHM, personal communication) wrote: "there is so little male-associated material that defining the genera on this sex just can't be achieved yet. As far as I can tell, *Strumigenys* and *Pyramica* cannot be separated on males." Xavier Espadaler determined that it was not a European species.

Unconfirmed Status (No Specimens Examined)

Lasius niger (L.)

Although Kirby (1884) listed *L. niger* from Bermuda, *Lasius* specimens from Madeira and the Azores, originally identified as *L. niger*, have been recently reclassified as *L. grandis* (Seifert, 1992), so the same may be true for the *Lasius* of Bermuda. It is also possible that the ants were not *Lasius* at all. Clark (1930) re-examined other ant specimens evaluated by Kirby and considered his identifications and descriptions to be "worthless." We did not find these specimens in the BNHM where Kirby worked.

Monomorium pharaonis (L.)

Wheeler (1906) speculated without examination that Verrill's (1902) *M. monomorium* specimens were actually *M. pharaonis*, a conclusion accepted by Ogilvie (1928) and Hilburn et al. (1990). Ogilvie (1928) wrote that *M. pharaonis* is a common house species, and Hilburn et al. (1990) wrote that it was "not an important household pest in Bermuda in recent years," but it is unclear whether either actually examined any *M. pharaonis* specimens from Bermuda.

Paratrechina sp.

Crowell (1968), Lieberburg et al. (1975), Haskins & Haskins (1988), and Hilburn et al. (1990) reported an unidentified *Paratrechina*. Hilburn et al. (1990) wrote that this species was "now common and widespread." However, all of the specimens from Hilburn et al. (1990) at the BAMZ labeled "*Paratrechina* sp." were actually *B. obscurior*. The same may be true of the other records.

Prenolepis sp.

Wheeler (1906) wrote that the *Prenolepis* sp. sample collected by Kincaid included "seven workers, apparently all from the same colony, but varying much in size (from 2-3 mm). They are very pilose and pubescent, with subopaque surface and finely punctate mesonotum." Wheeler (1906) felt that males were needed for definitive identification. We did not find these specimens at the MCZ, where Wheeler worked. It is possible that this species is a *Paratrechina* or perhaps a *Plagiolepis*.

Tetramorium caespitum (L.)

Verrill (1902) recorded *T. caespitum*, the European "pavement ant," in Bermuda. Wheeler (1906) speculated, without examining the specimens, that they were *T. guineense*. *Tetramorium caespitum* is common in Europe and Asia, as well as in the Azores. *Tetramorium guineense* is native to Africa, though Wheeler (1906) was no doubt actually referring to *Tetramorium bicarinatum* (Nylander), a common tramp ant once considered a synonym of *T. guineense*. Unfortunately, we did not find any of Verrill's specimens in the YPM, where he worked.

Ants Intercepted by Bermuda Department of Agriculture

Several species of ants in the BDOA collection were intercepted on incoming products, including: *Camponotus floridanus*, *C. novoboracensis*, *C. pennsylvanicus*, *Camponotus* sp. near *pennsylvanicus*, *Camponotus zonatus*, *Crematogaster steinheili*, and *Pheidole moerens*.

Discussion

Our study confirms the conclusions of earlier research (Haskins & Haskins 1965, 1988; Crowell 1968; Lieberburg et al. 1975) that Bermuda is largely partitioned between two dominant ant species, *Pheidole megacephala* and *Linepithema humile*. Although *P. megacephala* appeared to show a resurgence in the late 1960s and early 1970s (Table 2, Haskins & Haskins 1988), *L. humile* now has the upper-hand, dominating most parts of the main islands of Bermuda. The recent populations of *P. megacephala* in Bermuda appear to be the lowest recorded. Still, this species persists in pockets on the main islands and on small islands not connected to the main islands. We found that *P. megacephala* dominated and *L. humile* was absent on two small islands, Nonesuch and Horn, with breeding population of cahow (*Pterodroma cahow*), an endangered endemic bird. The absence of *L. humile* is relatively good news for the cahow because *L. humile* seems to pose a greater threat to ground-nesting birds than does *P. megacephala*. For example, Newell & Barber (1913) observed *L. humile* attacking young birds, swarming over and devouring nestlings.

In additional to the two dominant species, we examined specimens of 18 other ant species from Bermuda. *Brachymyrmex obscurior*, though small and inconspicuous, is very common in Bermuda and coexists with both *L. humile* and *P. megacephala*. *Paratrechina longicornis*, which was not previously reported from Bermuda, has substantial populations in two urban areas. Three other ant species appear to be well established, but very inconspicuous due to their very small size (*Brachymyrmex heeri* and *Solenopsis* sp.) or subterranean habits (*Hypoponera opaciceps*). The rest of the recorded ant species appear to be rare. Only three ant species with confirmed records from Bermuda have not been collected recently (1987 or later): *Hypoponera punctatissima*, *Paratrechina vivid-*

ula, and *Wasmannia auropunctata*. All three are common tramp species and almost certainly exotic to Bermuda.

It is an open question as to whether Bermuda ever had any native ants. It is feasible that Bermuda, like Hawaii, had no ants before people arrived. In fact, 13 of the 17 confirmed ant taxa in Bermuda identified to species are almost certainly exotic. Candidate for native status include *Brachymyrmex heeri*, *Brachymyrmex obscurior*, *Hypoponera opaciceps*, and *Odontomachus ruginodis*, all native to the West Indies and the Bahamas. Some species may have had native populations augmented by subsequent human-assisted immigration.

DNA analyses should be useful in evaluating native versus exotic status of ants in Bermuda, e.g., to determine whether or not populations of *B. heeri*, *B. obscurior*, *H. opaciceps*, and *O. ruginodis* show the genetic uniformity consistent with exotic introductions. DNA analyses may also allow evaluation of the geographic origins of populations of exotic species. DNA analyses of 35 of our *L. humile* specimens (five each from seven populations) showed that all individuals had the same haplotype for two mitochondrial markers (cytb and COI). These haplotypes have been found in one native Argentine population and in one introduced Chilean population, and but in no other introduced populations analyzed (V. Vogel et al., unpublished data; see Giraud et al. 2002).

More thorough ant surveys of Bermuda would be valuable. Of the 20 ant species with confirmed records from Bermuda, five have been collected only once. From this, we expect that there are several additional undocumented ant species established in Bermuda. The impact of ants on the native fauna and flora of Bermuda also deserves careful study.

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EFFECT OF MICROMITE® ON THE EGG PARASITOIDS *CERATOGRAMMA ETIENNEI* (HYMENOPTERA: TRICHOGRAMMATIDAE) AND *QUADRASTICHUS HAITIENSIS* (HYMENOPTERA: EULOPHIDAE)

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The Diaprepes root weevil, *Diaprepes abbreviatus* (L.), was first detected near Apopka, Florida in 1964 (Woodruff 1964). Since then, it has spread throughout the citrus growing areas of the state causing growers millions of dollars in losses each year. In south Florida, *D. abbreviatus* is also a problem in root crops and ornamental plants (Peña & Amalin 2000). One of the components of the pest management program for this weevil is the use of Micromite® (diflubenzuron), a chitinase inhibitor that sterilizes the egg by interrupting the formation and deposition of chitin in developing embryos. Early studies indicated that diflubenzuron significantly reduces the reproductive potential of *D. abbreviatus* when applied to citrus foliage (Schroeder et al. 1976; Lovestrand & Beavers 1980; Schroeder et al. 1980). Later, Schroeder (1996) reported that residues of diflubenzuron significantly affected the reproductive potential of *D. abbreviatus* for more than one month after application to citrus foliage.

Micromite has been described as a foundation product for reducing citrus root weevil populations, in part due to its compatibility with root weevil natural enemies (Anonymous 1996). However, there are no reports on the effect of micromite on egg parasitoids that have been imported and established for biological control of *D. abbreviatus* (Hall et al. 2001; Peña et al. 2000; Peña et al. 2003). A study was initiated to evaluate the impact of Micromite on *Ceratogramma etiennei* Delvare and *Quadrastichus haitiensis* (Gahan), two egg parasitoids of Diaprepes root weevil.

Green buttonwood (*Conocarpus erectus* L.) seedlings were grown from cuttings in 3.7-liter pots. Adult *D. abbreviatus* were collected from an insecticide-free ornamental orchard in Homestead, Florida. A Guadeloupe strain of *C. etiennei* was obtained from J. Etienne, Institut National de la Recherche Agronomique (INRA). The culture was maintained at the Tropical Research and Education Center (TREC) insectary, Homestead, Florida. Insect cultures were maintained at 26.5 ± 1.0°C, 12:12 L:D and approximately 78% RH, on eggs of *D. abbreviatus* laid on strips of wax paper using the methodology of Etiennei et. al (1990*). Quadrastichus haitiensis* originally from Puerto Rico was obtained from Ru Nguyen, Division of Plant Industry, Gainesville, Florida, and maintained as above.

The effect of Micromite ingestion by the adult Diaprepes root weevil plus absorption of residues from leaves into eggs was evaluated in experiment 1. Three green buttonwood seedlings planted separately on 3.7-liter pots were sprayed with Micromite to run-off using the simulated field rate (0.485 g/1 liter of water). Another three seedlings were sprayed with water as control checks. All the seedlings were enclosed separately in screen cages (240 cm × 120 cm × 120 cm). One hundred adults of *D. abbreviatus* were introduced inside each cage for oviposition. After 3 days, 20 egg masses on leaves still attached to branches were collected from each seedling, arranged as bouquets in flasks of water and placed in Plexiglass cages (30 cm × 30 cm × 30 cm) separately. Six cages, 3 treated and 3 untreated, were prepared for each parasitoid species. One hundred 2- to 3-d-old *C. etiennei* and *Q. haitiensis* adults were introduced into each Plexiglass cage. Bouquets were removed after 3 days and portions of the leaves with individual egg masses laid between two leaf surfaces (covered with two leaf layers intact) were placed in culture tubes (12 mm × 75 mm). After 7 d, egg masses were exposed by removing one leaf surface and parasitized eggs counted. Parasitized eggs were recognized by the characteristic golden egg chorion for *C. etiennei* and silver transparent egg chorion for *Q. haitiensis* (Peña et al. 2000).

Effects of ingestion of micromite by adult *D. abbreviatus* alone were evaluated in a separate experiment. Ten adult *D. abbreviatus* females were exposed to Micromite -treated seedlings and to untreated foliage for 3 days as described above. The adults were allowed to oviposit on to double strips of wax paper inside Plexiglass cages (30 cm × 30 cm × 30 cm). After 2 d, paper strips containing 20 egg masses were collected from each cage and each egg mass was placed separately inside culture tubes (12 mm × 75 mm). One 2-d-old mated female wasp was introduced into each tube (20 tubes per treatment for each parasitoid species). After 7 d, eggs were examined as described above.

In a third test, 10 untreated female weevils were allowed to oviposit on wax paper for 2 days. Wax papers containing approximately 20 egg

TABLE 1. EFFECT OF MICROMITE EXPOSURE BY ADULT FEEDING ON TREATED FOLIAGE (INGESTION), OVIPOSITION ON TREATED SURFACE (FOLIAGE), OR BY DIPPING EGG MASSES IN PESTICIDE (DIPPED) ON *CERATOGRAMMA ETIENNEI* PARASITISM ON *D. ABBREVIATUS* EGG MASSES.

	Source of Micromite exposure*			Percent Parasitized	
Experiment	Female treatment	Substrate	Egg mass	Control	Treated
1	Ingestion	Treated (foliage)	None	78.6 a**	0.0 b
2	Ingestion	None (wax paper)	None	95.0 a	0.0 b
3	None	None (wax paper)	Dipped	40.0 a	30.0 a

*Treatments were applied prior to parasitoid oviposition and all egg masses were sandwiched two between layers of substrate throughout the experiments.

**Means in rows followed by the same letter are not significantly different according to DMRT.

masses were dipped for 30 sec in 0.485 g/1 liter Micromite suspension and 20 egg masses were dipped in water as control. The strips of wax paper were left in place leaving the eggs covered on both sides. Wax papers were air dried and individual egg masses were placed singly in culture tubes, exposed to parasitoids and evaluated as above.

The effect of direct sprays on *C. etiennie* pupae was evaluated in a grove with Hamlin orange (*Citrus sinensis* [L.] Osbeck) trees in Hendry County Florida. Treatments were (1) Micromite 80 WG@ 5 oz ai per acre (6.25 oz/acre) plus 1% F433-66 horticulture oil, (2) F433-66 horticulture oil only @ 5%, and (3) untreated control. Plots consisted of single rows, each with 20-23 trees separated by 4 guard rows. Wax paper strips containing *D. abbreviatus* eggs exposed to *C. etiennei* 12, 14, and 16 days earlier and thus containing parasitoid pupae, were stapled on leaves. There were 22 parasitized egg masses of each age group within each of the 3 treatments. Trees were sprayed on 27 September 2000 from both sides using a Durand Wayland 3P 100-32 airblast speed sprayer equipped with 3 nozzles #3 T-Jet stainless steel nozzles, operating at 400 psi and calibrated to 91 GPA. Wax papers were collected the following day and allowed to incubate in the laboratory as above. Emerged wasps were counted.

Mean percent parasitization was computed and compared by Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at $P = 0.05$.

No successful development of *C. etiennei* embryos was observed in egg masses from female *D. abbreviatus* fed on Micromite-treated seedlings, whether oviposition occurred on leaves or wax paper (Table 1). Although, there was no significant difference ($df = 39, 1, F = 3.23, P = 0.08$) between Micromite-treated egg masses and the untreated control in emergence of *C. etiennei*, a trend toward less emergence from treated egg masses was observed. In contrast, no reduction of *Q. haitiensis* emergence was observed in response to host feeding on treated leaves ($df = 39, 1, F = 0.02, P = 0.87$), nor in response treatment of egg masses ($df = 39, 1, F = 2.44, P = 0.12$) compared to untreated controls.

In the field experiment, both Micromite and oil sprays reduced emergence of *C. etiennei*, although the influence of parasitoid age depended on the treatment (Table 2). Oil had its greatest impact on the older parasitoids (treatment 14 and 16 d after parasitization) with no significant effect on eggs parasitized 12 d prior to treatment. This might be due to a greater effect of suffocation on older parasitoids presumably respiring at a higher rate. In contrast, Micromite had its greatest impact on the younger parasitoids (initiated 12 and 14 d prior to treatment). No significant effect was observed on emergence from eggs parasitized 16 d before treatment, presumably because parasitoids had already synthesized sufficient chitin to complete development. This result confirmed the sensitivity of *C. etiennei* to Micromite, particularly of younger parasitoids.

These results suggest that Micromite interferes with development of *C. etiennei* but not *Q. haitiensis* in *D. abbreviatus* eggs. Furthermore, the effect on *C. etiennei* is age dependent, with greatest impact on young (developing) parasitoids and young pupae as compared to old pupae. This incompatibility may be one of the reasons for the continuing recovery of *Q. haitiensis* in south Florida in contrast to *C. etiennei* (Peña et al., un-

TABLE 2. PERCENT EMERGENCE OF *CERATOGRAMMA ETIENNEI* AT DIFFERENT AGE AT TIME OF TREATMENT (LENGTH OF INTERVAL FROM PARASITIZATION TO EXPOSURE IN THE FIELD).

	% Emergence by parasitoid age (d)		
Treatment	12	14	16
Micromite	0.62 b	1.48 b	7.27 ab
Oil	2.86 ab	5.09 b	4.42 b
Untreated	8.19 a	13.01 a	14.10 a

Means in columns followed by the same letters are not significantly different ($P \leq 0.05$).

published data). Although Micromite is known to be relatively safe to beneficial insects, *C. etiennei* proved to be highly sensitive. Thus, extensive use of this pesticide to control *D. abbreviatus* could be at least partially responsible for difficulties experienced in establishing *C. etiennei* (Hall et al. 2001). Perhaps more successful biological control could be achieved in the absence of this pesticide.

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Summary

This study reports the impact of Micromite on parasitization of *D. abbreviatus* egg masses by the parasitoids *Ceratogramma etiennei* and *Quadrastichus hatiensis*. *Diaprepes abbreviatus* egg masses treated with Micromite resulted in lower egg parasitization by *C. etiennei*. Micromite did not appear to affect egg parasitization by *Q. hatiensis*. A field test confirmed the sensitivity of *C. etiennei* to Micromite as well as to horticulture oil.

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FUNGAL PATHOGENS OF THE GLASSY-WINGED SHARPSHOOTER *HOMALODISCA COAGULATA* (HOMOPTERA: CICADELLIDAE)

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The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), is native to the southeastern United States, but was reported in California in 1990 (Sorensen & Gill 1996). This homopteran pest is now widely established in southern California (Blua et al. 1999), and it is also present in northern and central California (Phillips 1998). The sharpshooter feeds and reproduces on an extremely wide variety of plants (over 130 plant species) (Phillips 1998).

The major problem associated with the sharpshooter is its ability to vector and transmit several strains of the pathogenic bacterium, *Xylella fastidiosa* (Well & Raju), that cause diseases in many plants. One of the most important of these infections is Pierce's disease of grapevines. Bacterial pathogens clog the xylem, and xanthum gum production exacerbates water stress in susceptible hosts. Infected plants display scorch-like symptoms before dying of dehydration. This vector is a serious new threat to vineyards because it moves faster and further into vineyards than other known vectors (Phillips 1998).

Chemical control with pyrethroids and neonicotinoids looks promising against immature and adults, but it is associated with residue contamination and interferes with biological control strategies (Varela et al. 2001). The most commonly used compounds for protecting *Xylella*-susceptible plants is imidacloprid, which is registered for home and landscape, and for use on non-food crops. Insecticidal soaps and oils are the least toxic and disruptive chemical compounds to biological control, but these chemicals are only effective against soft-bodied nymphs of the sharpshooter (Varela et al. 2001).

Biological control methods are being investigated to reduce populations of the sharpshooter. Currently, the control strategies focus on several natives and imported egg parasitoids from South America. The most important parasite released against the sharpshooter is *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). Although the rate of egg parasitism by *G. ashmeadi* can reach 80% in Florida and Louisiana, and up to 100% in California (Phillips 1998; Triapitsyn et al. 1998), large numbers of the sharpshooter remain throughout the season (Varela et al. 2001).

Very little is known about predators, parasitoids, and pathogens that attack the sharpshooter nymphs and adults. We examined specimens of several populations of the glassy-winged sharpshooter where an epizootic of fungal infections were widespread in the fall of 2002 in Poplarville, Mississippi. We collected samples of about 30 glassy-wing sharpshooter cadavers that had died on crape myrtle (*Lagerstroemia indica* L.) and holly (*Ilex myrtifolia* Walter) for identification of the pathogens. The fungus found on some cadavers of sharpshooters bearing conidiophores was identified to be *Trichothecium roseum* (Pers.) Link. Several other specimens were covered with *Beauveria bassiana* (Bals.) Vuillemin.

To confirm the identification of the primary pathogen, another set of twelve specimens of glassy-winged sharpshooters collected was surface-sterilized by dipping them successively in 65-70% ethanol (10-15 min), 2% sodium hypochlorite solution (2-3 min), and sterile water (20-40 seconds). Smears or whole specimens were then plated on Petri plates (10.0 × 1.5 cm) containing either oatmeal agar or Sabouraud maltose agar (Difco, Detroit, MI) supplemented with 1% yeast extract (SMAY), and incubated at 27 ± 1°C, 85% RH for 7-14 days. After incubation, both fungi, *T. roseum* and *B. bassiana* were not found on the Petri plates, suggesting these fungi probably invaded the specimens after they were killed by another fungal pathogen. Madelin (1996) reported that *T. roseum* is not a virulent pathogen of many insects while *Trichothecium acridiorum* (Trabut) Madelin appears to be the main entomogenous species of this genus (Madelin 1996). Both of these species grow and sporulate relatively easily on a wide range of media and should probably be best regarded as nonfastidious saprobes. Similarly, the fungus, *B. bassiana* occurs worldwide but its main natural hosts are usually Lepidoptera, Coleoptera, Hemiptera, Diptera, and Hymenoptera rather than Homoptera, although

B. bassiana can affect a wide range of homopteran hosts (Li 1988).

Further, after incubation of the inoculum from the white ball of conidia from the cadavers of the glassy-winged sharpshooter, we found that the primary fungal pathogen is a species in the genus *Pseudogibellula* (Samson & Evans 1973). The morphology of the conidiophores is similar to those of *Pseudogibellula formicarum* (Mains) Samson & Evans (1973); there is a roughened stalk tapering towards the top end and forming a slightly bulbous swelling at the apex that then bears nearly globose cells in one or two layers. These cells bear a single, spherical outer layer of conidiogenous cells.

The overall characteristics of this species from Mississippi indicate that the fungus is *Pseudogibellula formicarum* (Mains) Samson & Evans (1973). In addition, we have isolated recently *P. formicarum* from dead glassy-winged sharpshooters collected from Weslaco, TX and tested the fungus for pathogenicity. Further, virulence and pathogenicity of *P. formicarum* to homopterans in the Ricaniidae and Cercopidae, mymicine and ponerine ants, spiders, and lagriid beetles has been reported (Samson & Evans 1973).

To determine the pathogenicity of *P. formicarum* (ARSEF, Ithaca, NY) to the sharpshooter, we cultured the fungus on SMAY, and incubated the Petri plates at 27 ± 1°C, 85% RH, and 13:11 (L:D) h photoperiod. We collected 14-day-old cultures and used them in each experiment. We also tested 14-day-old cultures of *Metarhizium anisopliae* (Metschinkoff) 5630 (EcoScience, New Brunswick, NJ) against the sharpshooter. Conidia concentration was determined with a hemocytometer. Both fungi were serially diluted in a solution of 0.02% Silwet L-77® (Loveland Industries Inc., Greeley, CO) to provide the concentrations needed for the bioassays.

Pseudogibellula formicarum was tested at concentrations of 2×10^8 and 2×10^9 conidia ml^{-1}, and *M. anisopliae* was tested at a single concentration of 2×10^8 conidia ml^{-1}. For each concentration, 5-7 sharpshooters were transferred to a glass Petri plate containing an ice-cold (4°C) and wet Whatman filter paper (90 mm diameter). The sharpshooters were sprayed with 1 ml of the conidial suspension in a Potter Precision Spray Tower (Burkhard Manufacturing, Rickmansworth, England) with 0.7 kg cm^{-2} pressure and a 0.25 mm orifice diameter nozzle. There were two replicates per fungal concentration and sharpshooters treated with deionized water containing 0.02% Silwet L-77® served as controls. After being sprayed, the sharpshooters were transferred to young seedlings of Cowpea (*Vigna unguiculata* L.) as food source. The seedlings were placed in Plexiglass cages (46 × 46 × 46 cm) (BioQuip Products, Gardena). The cages were maintained at 27 ± 2°C, 85 ± 2% RH in the greenhouse and in the Percival Scientific incubators (Percival Manufacturing Company, Boone, IA).

To determine conidia viability at the time of each experimental run, each concentration of fungal suspension was sprayed onto three Petri dishes containing SMAY. The conidia were incubated for 20 h at 27 ± 1°C, 85% RH. After incubation, three droplets of lactophenol cotton blue stain (0.5% cotton blue) were added to each Petri dish to fix and stain the conidia, preventing any further germination from occurring in the sample. The droplets were covered with a glass slide and evaluated with 400× phase-contrast magnification. The number of conidia that germinated in the first 100 conidia observed under the microscope was determined for each of the three droplets on each slide.

Dead sharpshooters were collected daily from the fungal treatments and the controls, and tested in the following way to determine if mortality was due to infection. The sharpshooters were surface-sterilized as described above. They were then transferred with a camel-hair brush to Petri dishes containing SMAY and incubated at 27 ± 1°C, 85% RH for 7-14 days. The Petri dishes were sealed with parafilm prior to incubation and the dead sharpshooters were observed daily for the presence of external fungal hyphae. The number of dead sharpshooters with external hyphae was counted, and to reduce the possibility of cross contamination, these sharpshooters were removed from the Petri dishes. Only sharpshooters that showed fungal growth were considered to have died of infection and used to compute the pathogenicity of the fungal pathogens.

Mean conidial germination was 97.6 ± 0.5% for *P. formicarum* and 98.6 ± 0.5% for *M. anisopliae.* Dead sharpshooters collected daily from the control samples showed no infection by *P. formicarum* after 21 days experimental period. In contrast, sharpshooters treated with 2×10^9 spores ml^{-1} and held in the incubators showed infection by the fungus ranging from 66% at day 7 to 93% 21 days posttreatments (Table 1). In greenhouse tests fungal infection ranged from 25% at day 7 to 81% at day 21 after the treatments were initiated (Table 1). A total of 76 dead sharpshooters collected from the treatment groups were investigated for fungal infection, and 70% of them showed mycosis at the end of 21 days of the experiments. Unlike the controls, the percentage of dead sharpshooters infected with *M. anisopliae* range from 20% at day 7, 50% at day 14, and 75% at 21 days after the fungal treatments (Table 1).

Overall, the glassy-wing sharpshooter was found to be suitable host for both *P. formicarum* (Figs. 1 and 2), and *M. anisopliae* (Fig. 3). However, a comprehensive study of virulence of the fungi has yet to be investigated. It appears that both fungal pathogens have the potential to control the glassy-wing sharpshooter.

TABLE 1. RECOVERY OF *PSEUDOGIBELLULA FORMICARUM* AND *METARHIZIUM ANISOPLIAE* FROM THE GLASSY-WING SHARPSHOOTER (GWSS), *HOMALODISCA COAGULATA* AFTER TREATMENTS.

Site	Treatments	N[a]	Infection of GWSS by *P. formicarum* after[b]		
			7 days	14 days	21 days
In Incubators	Control	15	0%	0%	0%
	2×10^8	15	17%	60%	75%
	2×10^9	20	66%	91%	93%
In Greenhouse	Control	12	0%	0%	0%
	2×10^8	13	10%	33%	48%
	2×10^9	15	25%	52%	81%
			Infection of GWSS by *M. anisopliae* after[b]		
			7 days	14 days	21 days
In Incubators	Control	10	0%	0%	0%
	2×10^8	13	20%	50%	75%

[a]Number of glassy-wing sharpshooters tested.
[b]Dead sharpshooters collected daily from treated and control samples were surface-sterilized, plated onto Petri dishes containing SMAY, and incubated at 27 ± 1°C, 85% RH to investigate the recovery of the fungi.

SUMMARY

An epizootic of fungal diseases on glassy-winged sharpshooters was examined in the fall of 2002 in Mississippi. *Trichothecium roseum* and *Beauveria bassiana* were identified from the cadavers of the sharpshooter bearing conidiophores, but these fungi appeared to be either secondary pathogens or saprobes rather than primary pathogens of the sharpshooter. *Pseudogibellula formicarum* was determined to be the cause of the epizootics. However, additional studies are need to provide a better understanding of host-pathogen interactions, and identify the factors that enhance or limit disease increase in sharpshooter populations under natural conditions. In addition, the sharpshooter was found to be also a suitable host for *Metarhizium anisopliae.* Overall, *P. formicarum* and *M. anisopliae* could provide new avenues for the biological control of the glassy-winged sharpshooter and complement current control strategies.

Fig. 1. Mycelia of *Pseudogibellula formicarum* emerge from dead glassy-wing sharpshooters collected from the treated samples after 4 days incubation at 27 ± 1°C, 85% RH. The sharpshooters were surface-sterilized and plated on SMAY to investigate the recovery of the fungus.

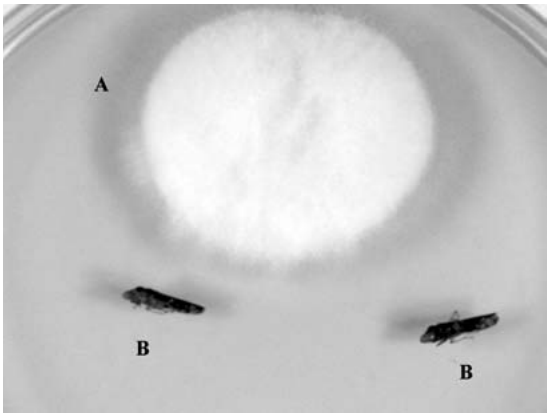

Fig. 2. A dead glassy-wing sharpshooter collected from the treated samples is covered with mycelia and conidia of *Pseudogibellula formicarum* (A), while control sharpshooters showed no fungal infection (B) after 7 days incubation at 27 ± 1°C, 85% RH. The sharpshooters were surface-sterilized and plated on SMAY to investigate the recovery of the fungus.

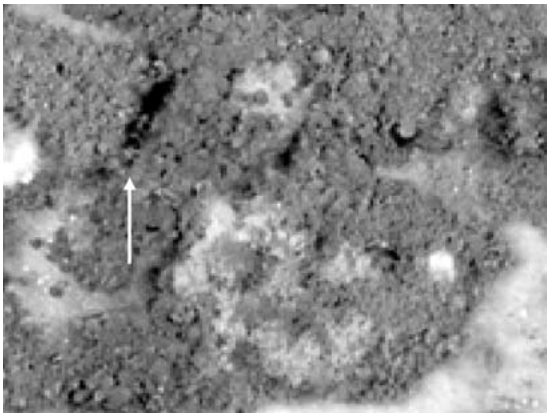

Fig. 3. Dry conidia of *Metarhizium anisopliae* emerge from dead glassy-wing sharpshooters collected from the treated samples after 14 days of incubation at 27 ± 1°C, 85% RH. The sharpshooters were surface-sterilized and plated on SMAY to investigate the recovery of the fungus.

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AGENIASPIS CITRICOLA (HYMENOPTERA: ENCYRTIDAE) ESTABLISHED IN BERMUDA

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The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was first found on November 21, 1998 and rapidly colonized all citrus trees in the 53.3 square km that comprise the islands of Bermuda (Jessey-Aldrich 1999). Management of the citrus leafminer with pesticides or cultural controls soon was found to be difficult and expensive, in part due to the biology of the pest; citrus leafminer females deposit eggs singly upon tender young citrus foliage (flush) and the larva then enters the leaf where it feeds on epidermal cells, producing broad serpentine mines in the leaves (Heppner 1993). High densities of *P. citrella* result in leaves that are twisted and damaged, have reduced rates of photosynthesis and, under severe conditions, may defoliate. Only a few parasitoids were found attacking the citrus leafminer at very low rates (<1%) after its arrival in Bermuda; these were identified as the eulophids *Closterocerus cinctipennis* Ashmead and *Pnigalio minio* Walker (C. Jessey, unpubl.).

Because leaves often contained more than five to six mines on their new growth, a classical biological control program was initiated by the Bermuda Department of Agriculture and Fisheries in January 2000 and the encyrtid *Ageniaspis citricola* Logvinovskaya was imported into Bermuda from a laboratory culture maintained in Florida (Smith & Hoy 1995). *Ageniaspis citricola* had established rapidly in Florida and performed well under Florida's humid subtropical conditions (Hoy & Nguyen 1997; Yoder & Hoy 1998).

Ageniaspis citricola is a koinobiont endoparasitoid and is polyembryonic, producing between one and ten progeny per leafminer host, averaging three (Hoy & Nguyen 1997; Edwards & Hoy 1998). It appears to be specific to the citrus leafminer, having been reared from very few other hosts (Hoy & Nguyen 1997). Parasitoid females attack eggs and small larvae of the leafminer and the developing leafminer pupates before the parasitoid completes its development within the host's pupal chamber.

Prior to releases of *A. citricola*, citrus trees were monitored throughout Bermuda in November 1999 to estimate the relative abundance of citrus leafminers on young flush. The survey indicated that the pest had colonized the entire island and all flush was infested with as many as six mines per leaf. Approximately 600 *A. citricola* were reared in the laboratory at the University of Florida in Gainesville (Smith & Hoy 1995) and shipped to the Bermuda Department of Agriculture and Fisheries as pupae. Pupae were held in the laboratory in plastic bags and emerging adults aspirated out for releases on January 25-27, 31 and February 2-10, 2000. Releases were made at eight sites into citrus trees containing young foliage and abundant citrus leafminers.

In April 2000, citrus trees were monitored to determine the number of mines and the proportion of pupal chambers that contained the citrus leafminer or *A. citricola* and *A. citricola* was already present in the majority of release sites surveyed (data not shown). Another survey was conducted in July 2002 at release and nonrelease sites (19 total) (Table 1). During this survey, intact pupal chambers were opened and the presence of leafminer or *A. citricola* pupae was recorded. An average of 94.3% of all pupal chambers contained *A. citricola* in July 2002. In some sites, fewer than 20 pupal chambers could be sampled because leafminer densities were very low in all trees sampled, always averaging fewer than one leafminer per leaf. Examination of older flushes during this survey indicated that, on average, the leaves on the previous flushes contained fewer than one mine per leaf, as well. The survey data suggest that citrus leafminer populations are lower than prior to the release and establishment of *A. citricola* and homeowners contacted during this 2002 survey indicated that most have discontinued pesticide applications for the citrus leafminer. This work was supported in part with funding from the Bermuda Department of Agriculture and Fisheries and the Davies, Fischer and Eckes Endowment in Biological Control. This is Florida Agricultural Experiment Station Journal Publication R-09858.

SUMMARY

Ageniaspis citricola has overwintered and dispersed since its release in humid subtropical Bermuda in 2000 and was found in all citrus trees sampled in 2002. It has become an important natural enemy of the citrus leafminer in Bermuda.

TABLE 1. SURVEY OF BERMUDA DURING JULY 2002 FOR ESTABLISHMENT AND RELATIVE ABUNDANCE OF *AGENIASPIS CITRICOLA* AND ITS HOST, THE CITRUS LEAFMINER. ATTEMPTS WERE MADE TO EXAMINE 20 PUPAL CHAMBERS FOR PRESENCE OF THE LEAFMINER *OR A. CITRICOLA* PUPAE AT EACH SITE BUT DENSITIES WERE SO LOW THAT FEWER WERE SAMPLED AT SOME.

Date 2002	Location/Parish	No. pupal chambers opened	% parasitized by *A. citricola*	Release site yes/no
7/26	1. Glasglow Lodge/Southampton	20	90	yes
	2. Newstead Hotel/Paget	9	89	no
	3. White Lodge/Pembroke	20	95	no
	4. Shaw Park/Pembroke	20	95	no
7/29	5. Government House/Pembroke	20	100	no
7/29	6. Crestwood/Paget	21	86	yes
7/29	7. Tungate/Paget	20	95	yes
7/29	8. Botanical Gardens/Paget	10	70	yes
7/30	9. Malpas House/Somerset	21	100	no
7/30	10. Old School Lane/Somerset	13	100	no
7/30	11. Parapet/Somerset	20	100	no
7/30	12. Chapel/Ease/St. Davids	22	91	no
7/30	13. Glen Duror/St. Georges	19	100	no
7/31	14. Hexham/Hamilton	21	95	no
7/30	15. Knapton House/Hamilton	2	100	no
7/30	16. Honey House/Hamilton	22	100	no
7/31	17. Windy Bank/Smiths	20	95	no
7/31	18. Sleepy Hollow/Hamilton	20	90	yes
7/31	19. Coral Beach/Paget	20	100	yes

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A NON-DESTRUCTIVE, AUTOMATED METHOD OF COUNTING SPORES OF *OPHRYOCYSTIS ELEKTROSCIRRHA* (NEOGREGARINORIDA: OPHRYOCYSTIDAE) IN INFECTED MONARCH BUTTERFLIES (LEPIDOPTERA: NYMPHALIDAE)

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Monarch butterflies (*Danaus plexippus*) are parasitized by the neogregarine protozoan parasite *Ophryocystis elektroscirrha* in varying levels throughout North America. In the eastern population, less than 8% of individuals are heavily infected, whereas in the western population, approximately 30% are heavily infected. Moreover, more than 70% of monarchs from the non-migratory South Florida population are heavily infected (Altizer et al. 2000). In Florida, the related queen butterfly (*D. gilippus*) is also susceptible to this parasite (McLaughlin & Myers 1970; Leong et al. 1997). The life cycle of *O. elektroscirrha* is such that its spores are transmitted by ingestion by larvae, vegetative reproduction occurs in the larvae and pupae, and the adult monarch emerges with spores covering the surface of its abdomen (McLaughlin & Myers 1970). Depending on the stage of larval growth at the time of exposure, the age and number of spores, infected adults can harbor from 200 to 974,000 spores on their abdomens (Leong et al. 1997). Furthermore, depending on the parasite loads, infected adults have decreased survival to eclosion, are subsequently smaller as adults, and have shorter adult lifespans (Leong et al. 1992; Altizer & Oberhauser 1999).

The susceptibility of monarchs to this parasite and the ease at which monarchs can be reared and inoculated with its spores has provided researchers with a model system for examining host-parasite relationships (e.g., Leong et al. 1992; Altizer et al. 1997; Altizer & Oberhauser 1999; Altizer 2001). In most of these studies, researchers are required to estimate the degree of infection in individual monarchs, which is inferred by the number of spores present on its abdomen, where they are known to be most numerous (Leong et al. 1992). To sample and count these spores, two general methods have been employed most commonly in the past. In the first method, the abdomens of adult specimens are removed, placed in a vial with water and a wetting agent, shaken on a vortexer, and the number of spores per volume of suspension is counted with a haemocytometer (Leong et al. 1992; Leong et al. 1997). Viewing and counting the spores in the haemocytometer is effective because the spores are dispersed throughout the solution, facilitating counting of individual spores. However, this method requires that monarchs be dead prior to quantifying parasite loads.

A non-destructive, simpler method is widely used by butterfly breeders and researchers to detect and remove parasitized individuals in their captive stock. It involves pressing a small piece of transparent tape over the abdomen of the live monarch, pulling it off to remove a section of abdominal scales, placing it on a microscope slide and viewing it under low power (e.g., Altizer et al. 2000; Altizer 2001). Spores of *O. elektroscirrha* are readily seen and counted among the butterfly scales at 50× or greater magnification. Unfortunately, when actual counts of individual spores are required, this method is tedious because there can be hundreds of thousands of spores that must be counted manually. Furthermore, since the tape removes many scales with the spores, the resulting slide contains dense clusters of butterfly scales that make it hard to discern the parasite spores.

Advances in photographic and computer technology during the past 15 years have led to the development of image analysis techniques. These techniques involve taking a picture of one or more objects, then transferring the picture to a computer, where digital measurements are made of the objects in the picture with special graphical software. This technique has many applications, spanning a multitude of disciplines. We used a variation of this technique to develop a method to quantify spores of *O. elektroscirrha* on adult monarch butterflies in a recent study, and we describe this method in the present paper. This method is objective, non-destructive, and once the samples are obtained, the counting process can be fully automated.

To obtain samples of the spores, we first held the adult butterfly by the base of its wings so that its abdomen was exposed. Next, we used a standard cotton swab to gently swipe one side of the abdomen with the tip of the swab from the posterior end forward, so that at least 4 abdominal segments were swiped (usually segments 4-8). We repeated this 4 times using the same swab in the same place on the abdomen. The end result was a small accumulation of abdominal scales and spores on the tip of the swab. Next, we lightly dabbed the swab tip on a dry, standard micro-

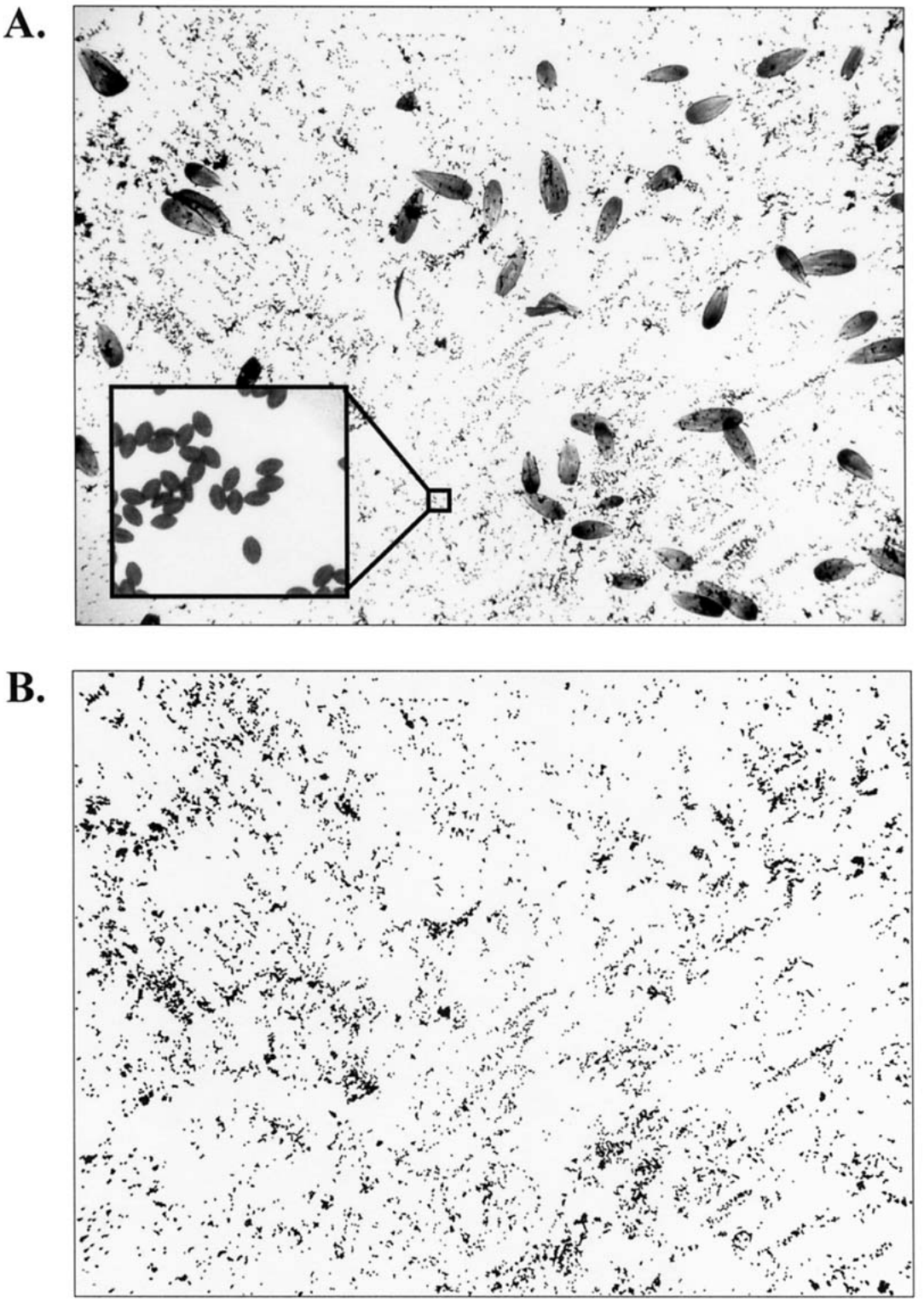

Fig. 1. (A) Image of *O. elektroscirrha* spores from a heavily infected monarch butterfly (40×) before image processing. Insert shows spores at 400×. (B) Same image after digitally deleting the scales to leave only spores for automated image analysis counting. Approximately 3,600 spores were counted in this sample.

scope slide 4 times, leaving a small circle of scales (and spores) which adhere to the dry slide. Then, we viewed the slide under 40× magnification with a light microscope equipped with a trinocular head and digital camera (Olympus C-3000 Digital Zoom). We positioned the field of view so that it was centered on the entire cluster of scales. In infected individuals, spores of *O. elektroscirrha* can be seen readily among the scales (Fig. 1A). We photographed this image with our digital camera at 3 megapixel resolution. This process was performed twice for each butterfly, once for each abdominal side, to reduce the chances of a poor swipe with the swab. Two images per butterfly were thus obtained.

To count the spores in the images, we used Adobe Photoshop with the Image Processing Tool Kit (Reindeer Graphics, Inc.) plugin installed. Before we began the counting procedure, we first calibrated the software by digitally drawing a line of a known distance on an image of a haemocytometer grid (which is 1 mm^2). Once the pixel-to-distance ratio in this image was saved, it was then possible to obtain correct sizes for any objects in all images thereafter. For each image of spores and scales, we then performed a series of steps to remove the butterfly scales from the image based on their size, and threshold the image (i.e., change the image to black and white) so that all remaining objects (spores) were black on a white background (Fig. 1B). It was then a simple matter to have the computer measure the area encompassed by black objects (spores) in the image. Because we previously found that the average area of an individual spore is approximately 45 μm^2, we divided the total spore area we obtained by the known individual spore area to arrive at the approximate number of spores in the image. In Fig. 1B, we obtained an estimate of 3,600 spores.

For greater accuracy, we calculated the average of the two counts (from separate swabs of the left and right sides of the abdomen) obtained for each butterfly. This resulted in an estimate of spore load that is comparable to manual counting from tape samples. As a test of this point, we sampled and counted spores on a set of 31 infected specimens by both the image analysis method and the tape method (i.e., by manually counting the number of spores on 1cm^2 tape samples at low power to the nearest 100 spores). Both methods were significantly positively correlated (e.g., for log-transformed values, r = 0.76, N = 31, $P < 0.001$; Fig. 2). Further, because we obtained two samples (from the left and right side of the abdomen) from each butterfly, we compared the repeatability of our method by testing whether the samples obtained from each side differed. The number of spores obtained from the left side of all butterflies differed from that obtained from the right side by an average of 478 (± 334, SD). There was a significant positive correlation between the counts obtained from the left and right sides for each individual monarch (Pearson correlation, N = 31, r = 0.65, $P < 0.001$).

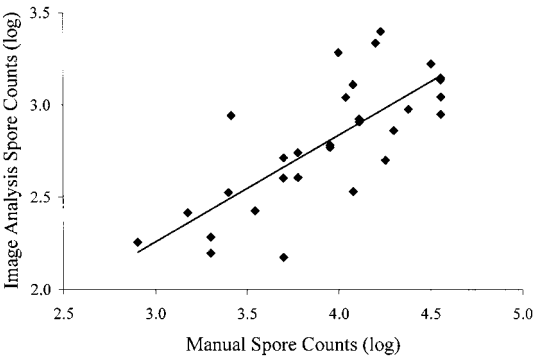

Fig. 2. Plot of manual spore counts of 31 adult monarch butterflies made from the manual (tape) method against spore counts obtained by the image analysis method for the same individuals (both counts are log-transformed). Counts obtained from both methods were significantly correlated (r = 0.76, $P < 0.001$). Least squares regression line shown (y = 0.5195 + 0.5795x).

In terms of assessing parasite loads on infected, live monarchs, this 'swab method' combines both the non-destructiveness of the tape method and the clear images of spores in the haemocytometer method. Furthermore, this method produces a fast, non-destructive, quantitative estimate of infection levels of individual butterflies. The only drawback of the method that we can perceive is its relative inability to detect minute spore loads (i.e., less than 100 spores on the abdomen). However, the advantages of the method may outweigh this one weakness. One unique advantage is the fact that for each butterfly sampled with this method, a permanent image of its spore load is created that can be saved and archived. Moreover, if image analysis software is not available to the researcher, the images can be easily emailed to persons capable of performing the counting procedure. Finally, with automation routines common to many software programs such as Adobe Photoshop, the counting procedure can be set up to run on multiple images either in the background of the desktop, or while the computer is not being used. The speed of the procedure will vary with the speed of computer used. In 30 minutes our software can obtain counts of spores on 50-60 images, with typical counts ranging from 300 to 12,000 spores in infected butterflies.

Summary

Monarch butterflies are susceptible to the protozoan parasite, *O. elektroscirrha*, and they have been the subject of study by researchers interested in host-parasite interactions (e.g., Leong et

al. 1997; Altizer et al. 2000; Altizer 2001). As such, methods have been developed to sample individual butterflies for the presence and severity of this parasite, but past methods are either destructive or tedious with respect to providing quantitative (non-categorical) measures of actual parasite loads. We developed a novel method for sampling and counting *O. elektroscirrha* spores on monarch abdomens based on image analysis techniques. This method is simple, non-destructive, and objective, and will provide a useful and effective tool for future researchers of this parasite.

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ORANGE TORTRIX, "*ARGYROTAENIA CITRANA*": A WESTERN SPECIES NOT IN FLORIDA (LEPIDOPTERA: TORTRICIDAE)

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The orange tortrix, *Argyrotaenia citrana* (Fernald), a citrus pest from California, is not known east of citrus regions of Arizona and has never been in Florida. A number of published reports maintain the erroneous observation that this species has or does occur in Florida as a pest on citrus, including recent papers by Bullock et al. (1997) and Razowski (2000). Reports of this pest in Florida have a major impact on citrus exports for the state, thus these erroneous reports can cause great economic havoc and need to be corrected. These reports are all based on early misidentifications of Florida specimens. All reported records of *A. citrana* in Florida have been checked and specimens examined, and all such reports have been verified to actually pertain to a native Florida species, *Argyrotaenia ivana* (Fernald), the ivana tortrix.

Orange tortrix only occurs along the Pacific Coast as far north as Washington and into southern Arizona, feeding on various plants such as apple and apricot trees in the north. One extraneous record for the species is also known from Michigan (Powell, pers. comm.), but this may be in error or only a specimen transported east with apple trees. The common name is misleading, since the main hostplants are not orange trees. However, in contrast to its usual use of northern fruit trees as larval host, in California and southern Arizona it also sometimes causes damage to citrus leaves as an alternate host, thus providing the reason for the common name misnomer, "orange tortrix". Weires and Reidl (1991) have given perhaps the best overall status report on this minor pest of citrus in recent years, also noting the above distribution and with no mention of Florida. Recent DNA studies (Landry et al. 1999), however, confirm that *A. citrana* is part of a complex of named forms and related species that should all refer to a single western species with the senior name of *Argyrotaenia franciscana* (Walsingham).

In Florida, the first report of what was called "orange tortrix" appears to be by Thompson (1939) in a short citrus pest summary in the 1938 Annual Report of the Florida Dept. of Agriculture. Numerous groves were damaged in 1938 in Florida (the exact locations are not stated and presumably in various commercial or experimental citrus groves) by a leafroller identified as the orange tortrix, *A. citrana*. Thompson (1939) does not state who made this identification.

Freeman (1944, 1958) made a complete study of the genus *Argyrotaenia*, including available specimens in major museums such as the Smithsonian Institution (USNM), in Washington, DC, and made no mention of orange tortrix in Florida. If Thompson had sent specimens to the USNM for identification, then Freeman would have found voucher specimens in the collections there during his later studies. The Florida State Collection of Arthropods (FSCA), Gainesville, Florida, which maintains the Florida collection of insects and also has all voucher specimens from the University of Florida and the Florida Dept. of Agriculture and Consumer Services, has no specimens dated 1938 that Thompson may have taken as samples. The Division of Plant Industry (DPI), which houses the FSCA collections and maintains all agricultural insect records for Florida, also has no card records for any identifications from 1938 for this species. One can only assume from this that Thompson, or someone else unfamiliar with Tortricidae, made the incorrect determination of the Florida specimens as the same as orange tortrix found in California.

The orange tortrix and the ivana tortrix appear similar superficially, so untrained observers would easily mistake one for the other. However, careful study of specimens, including genitalic characters, easily distinguishes the two species. While *A. citrana* may now be part of *A. francsicana*, both named species are western North American and differ from *A. ivana*.

Over the years, other specimens from Florida have been misidentified as orange tortrix in various unpublished citrus pest reports: all such records with DPI identification data and the accompanying FSCA specimens have been checked and all actually refer to the ivana tortrix. The erroneous records were all repeated in published works (Kimball 1965), and further repeated in citrus reports over the years. Bullock et al. (1997), while reporting on minor citrus feeding by two other *Argyrotaenia* species in Florida, also repeated the old reports of the presence of orange tortrix in Florida. Even a recent tortricid catalog (Razowski 2000) maintains the old error and again erroneously gives orange tortrix a distribution that includes Florida.

My own studies on the Florida Lepidoptera long ago showed that orange tortrix was not present in Florida, and this was indicated whenever any suspect orange tortrix were written about for Florida in agricultural reports. Likewise, it is noted in the new catalog of Florida Lepidoptera (Heppner 2003). The correct information on ivana tortrix in Florida also was published recently in a short note (Heppner 2001) to clarify

this fact, and the present paper further publicizes the fact that orange tortrix does not occur in Florida and never has.

Summary

Orange tortrix, known as *Argyrotaenia citrana* (Fernald) but now thought to be part of the western North American species *A. franciscana* (Walsingham), is noted to have never been recorded from Florida. All previous reports of this species for Florida are erroneous and actually refer to a similar species native to Florida, *Argyrotaenia ivana* (Fernald), the ivana tortrix.

Contribution No. 940, Entomology Section, Bur. Ent. Nema. Plant Path., Div. Plant Industry, Florida Dept. Agric. & Consumer Serv., Gainesville, FL.

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EUMICROTA AND *PHANEROTA* (COLEOPTERA: STAPHYLINIDAE: ALEOCHARINAE) ATTACKING CULTIVATED MUSHROOMS IN FLORIDA

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Most Staphylinidae, as adults and larvae, are predators or facultative predators. However, minorities feed only on plant materials including fungi. Among these minorities is the subtribe Gyrophaenina, which contains only fungivores. It belongs to the tribe Homalotini and subfamily Aleocharinae. Six genera of Gyrophaenina (Ashe 2001) occur in America north of Mexico, of which three (*Eumicrota* Casey, *Gyrophaena* Mannerheim, and *Phanerota* Casey) occur in Florida. Four species of *Eumicrota*, two of *Gyrophaena*, and five of *Phanerota* (Frank 1986) are known in Florida, although it is possible that some species are yet unreported.

Seevers (1951), despite using an out-of-date classification, gives the only recent keys to North American *Eumicrota* and *Gyrophaena* adults, including the species known in Florida. A checklist of Florida Staphylinidae (Frank 1986) includes mention of a new species description in *Phanerota* and key to adults of Florida species of that genus by Ashe (1986a).

There are thus 11 known species of Gyrophaenina in 3 genera in Florida. Adults of all species are small, ranging from 0.6 mm up to about 2 mm in length. Adults and larvae feed by grazing on spores, basidia, cystidea, and hyphae from the hymenium layer of fruiting-bodies (mushrooms). Their food range is limited to the Polyporaceae and several families of gilled mushrooms. Among the various species groups of *Gyrophaena*, Ashe (1984) detected some level of specialization to fungal hosts. However, earlier fungal identification is in doubt because most beetle collectors do not have such skills. The mushrooms of North America are described and illustrated by McKnight & McKnight (1987), but even expert mycologists encounter mushrooms in Florida that they find difficult to identify to the species level, in part because some are yet undescribed (K. H. McKnight, pers. comm.). No comparative study about the habits of species of Gyrophaenina occurring in Florida has been written. The subject would make a good research project for a graduate student interested in these beetles and in mycology.

Gyrophaenine staphylinids are not the only staphylinid beetles, and far from the only beetles, that feed on mushrooms. Some Staphylinidae attracted to mushrooms prey on other small insects. An entire book was published on beetles and mushrooms observed in the vicinity of Vienna, Austria (Scheerpeltz & Höfler 1948). A book with adequate worldwide treatment of beetles and mushrooms is still far in the future because so much is unknown.

Fruiting-bodies of gilled mushrooms provide an ephemeral habitat in nature, and are unpredictable in time and space. Fruiting-bodies of polypore mushrooms are less ephemeral. The physical and chemical characteristics of mushrooms are diverse. Gyrophaenines eat only fresh mushrooms, not those in decomposition. Constraints on the adult beetles are that they must be able to detect ephemeral fresh mushrooms efficiently, despite the varied physical form and chemical characteristics of the mushrooms. The eggs and larvae must develop rapidly before decomposition of the mushrooms occurs. Pupation occurs in a silken cocoon in the soil. The adults are potentially much longer-lived, and they are winged and can fly. It is the larvae that must eat intensively and rapidly in order to complete development quickly (Ashe 1984).

In March 2003, an organic grower of mushrooms in Lee County, Florida, noted an infestation of small insects on oyster mushrooms (*Pleurotus ostreatus* [Jacq.] Quélet, Tricholomataceae) grown indoors. Collected specimens were placed in alcohol for subsequent identification. Based on keys by Ashe (1986a) and Seevers (1951), these were identified as two *Eumicrota socia* (Erichson), four *Phanerota cubensis* Casey, one *P. fasciata* (Say), and an unidentified larva. More specimens were collected on 29 April, and these included four *E. socia*, ten *P. cubensis*, 36 *P. fasciata*, and six unidentified larvae.

Eumicrota socia was described from the Carolinas in 1840. In 1906 it was described under four new names in various states of the USA, and in 1920 it was described from northern Florida under yet another new name (Seevers 1951). We assume that this is a Florida native species in which minor intraspecific variability and lack of an adequate series of specimens confused early writers of descriptions, who failed to examine long series of specimens for intraspecific variability, and failed to dissect specimens and examine the microscopic sexual characters. Adults of *Eumicrota* exhibit a subsocial behavior in guarding eggs in an egg chamber (Ashe 1987), and the larva of one North American species was described by Ashe (1986b).

Phanerota cubensis, as the name implies, was originally described from specimens collected in Cuba, published in 1906. Ashe (1986a) reported it in localities from Dade and Monroe counties in

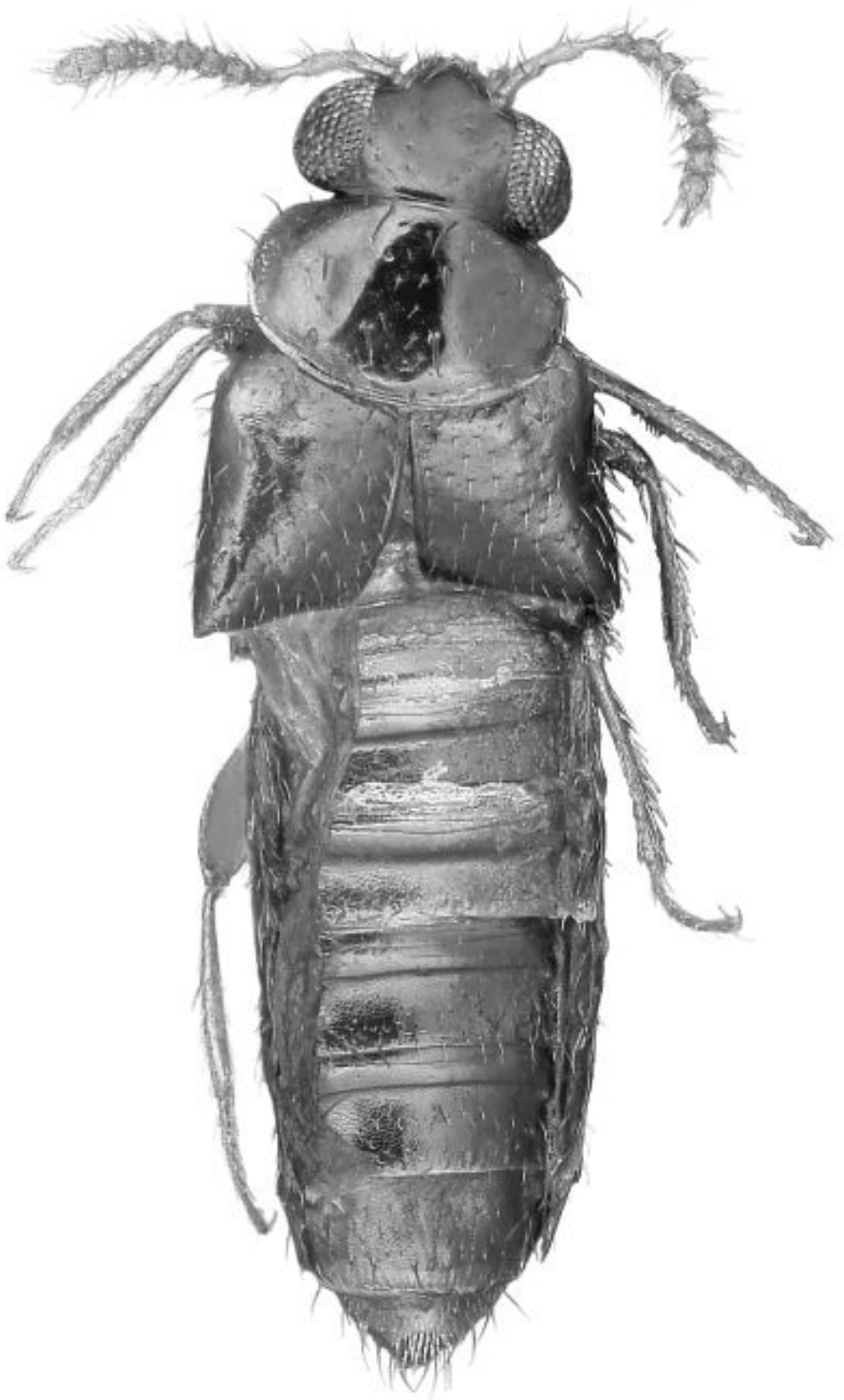

Fig. 1. Habitus photograph of adult *Phanerota fasciata*. The mark on the pronotum is a shadow, not an indentation.

Florida. We think it was not reported earlier from Florida simply because nobody looked extensively in mushrooms for tiny staphylinids in the extreme south of Florida until the 1970s, and nobody bothered to identify specimens of this species until the 1980s. We do not know how it got to Cuba from Florida or vice versa, but wind-assisted flight is the simplest explanation.

Phanerota fasciata (Fig. 1) was described in 1834 based upon specimens collected in Pennsylvania. The type specimens of other nominal species later declared to be synonyms of *P. fasciata* were collected in Florida, New York, and Texas; also, the species was found to be widespread in the eastern USA, as far west as Kansas and Texas (Seevers 1951). The larva of *P. fasciata* was described by Ashe (1986b).

None of these three species has previously been reported from Lee County, probably due to inadequate knowledge of these little beetles in Florida. The grower's question was about how to eliminate the insects from the mushroom culture. A search of the computerized literature (post-1970) revealed only one publication on an infestation of such beetles anywhere in the world. That publication (Shivaramu et al. 1993) revealed only an identification of *Gyrophaena nilambura* Cameron, which, along with non-staphylinid beetles, was found in mushroom cultures in India, but gave no indication about growing conditions, the extent of the problem, or control methods. It should be noted that *G. nilambura* does not occur in North America and is just one of well over 100 species of Gyrophaenina recorded from "British India including Ceylon and Burma" (Cameron 1939). This makes it highly improbable that *G. nilambura* is the only species that causes or may cause problems in the countries now called India, Bangladesh, Pakistan, Sri Lanka, and Myanmar.

Because the mushrooms in Lee County were grown indoors, it should have been fairly easy to control the infestation. The building is approximately 3.66 × 6.10 m (12 × 20 ft) and of concrete block construction, and its windows had USA-standard ("16 × 18 mesh") window screening, whose openings are ≈1.5 mm, thus larger than the maximal width (≤1 mm) of any of the tiny adult beetles; they could not fly in with wings extended, but they could alight on the screen and crawl in. Of course the little adult beetles would have been attracted to the cultures, which provided a permanent source of mushrooms (as contrasted with ephemeral and unpredictable sources in nature), and they would have entered the building by any means possible. We recommended (a) temporary elimination of all mushroom cultures from the building, (b) vacuum-cleaning of all deposits likely to house adult beetles, (c) a temporary trap in the building, to be constructed of mushrooms in a net bag suspended over a large pan of soapy water, to trap flying adults, (d) fitting of the windows with screen of very fine mesh, and fitting the entrance with double screen doors, (e) if employee time were sufficient, frequent inspection of the grounds for "wild" mushrooms and their hand-picking to eliminate local populations, and (f) compostion of rejected cultivated mushrooms as well as any hand-collected from the property, in plastic bags outdoors in the sun (because solar heat and the heat generated within the bags should kill any of these insects). We were later told that fitting of the windows with screen of finer mesh caused other kinds of problems; we presume this was a result of reduction of airflow. We recommend, that in future construction of such buildings, the window area should be greatly increased, and windows should be fitted with screen of very fine mesh in order to block entry of these beetles yet maintain an adequate airflow. The ideal design needs further study.

We acknowledge reviews of the manuscript of this note by Ronald D. Cave (Univ. Florida, Ft. Pierce, FL) and Michael C. Thomas (Florida State Collection of Arthropods, Gainesville, FL). Dr. Kent H. McKnight was, until his retirement, a mycologist at the USDA Beltsville Agricultural Research Center, Maryland. David Almquist (formerly a student in the Entomology & Nematology Dept., Univ. Florida, Gainesville, FL) kindly prepared the habitus photograph (Fig. 1). This is University of Florida, Agricultural Experiment Station, Journal Series No. R-09738.

Summary

We report what may be the first recorded infestation of cultivated mushrooms in North America by gyrophaenine Staphylinidae. The infested mushrooms were *Pleurotus ostreatus* (Jacq.) Quélet (Tricholomataceae), called "oyster mushrooms", and were grown organically indoors. The infesting beetles were *Eumicrota socia* (Erichson), *Phanerota cubensis* Casey, and *Phanerota fasciata* (Say); they are part of Florida's native "wildlife", and they normally eat uncultivated wild mushrooms and cause no problem.

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EVALUATION OF SUBTROPICAL AND TROPICAL FRUITS AS POTENTIAL HOSTS FOR THE SOUTHERN STRAIN OF PLUM CURCULIO (COLEOPTERA: CURCULIONIDAE)

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The host range of plum curculio, *Conotrachelus nenuphar* (Herbst), includes a variety of native and introduced rosaceous fruits in temperate North America, including *Prunus* spp. (stone fruits), *Crataegus* spp. (hawthorns), *Amelanchier* spp. (juneberries), and *Malus* spp. (apples) (Maier 1990). Larvae may also develop on rain-softened peach mummies caused by brown rot, *Monilinia fructicola* (Winter) Honey (Sarai 1969), or rosaceous plant tissue damaged by the diseases black knot, *Apiosporina morbosa* (Schw.) von Arx, or plum pockets, *Taphrina communis* (Sadelbeck) Giesenh. (Quaintance & Jenne 1912; Wylie 1966). The host range extends beyond the Rosaceae; plum curculio is an economic pest of blueberries, *Vaccinium* spp. (Ericaceae) and develops in gooseberries, *Ribes* spp. (Saxifragaceae) (Armstrong 1958). The pest has been observed to oviposit in a number of fruits in which larvae do not develop, and some rosaceous fruits are not hosts (Quaintance & Jenne 1912; Maier 1990). Adults prefer to oviposit in immature fruits that are a fraction of harvested size.

Two geographically overlapping strains of plum curculio are recognized; the northern is univoltine and undergoes obligate diapause, and the southern is multivoltine and undergoes facultative diapause. The two strains are somewhat reproductively incompatible (Padula & Smith 1971). Plum curculio is not found outside of its native geographical limits of North America east of the Rocky Mountains, save for a localized infestation of the northern strain in Box Elder County, Utah (D. Alston, pers. comm.). The southern limit of the plum curculio's range is about latitude 28 degrees north, which passes through mid Florida and southern Texas. These lowland areas are hot (>35°C) for several months of the year, which may be a factor in limiting its southern range. However, it is conceivable that the southern strain plum curculio might become established in more moderate tropical and subtropical regions of the world, particularly at higher altitudes.

The objective of this research was to offer southern strain plum curculio a broad taxonomic range of fruits found in the tropics and subtropics to gain insight into the possibility of it attacking other fruits if it became established in those regions.

Southern strain plum curculios were obtained from a colony at the United States Department of Agriculture, Agricultural Research Service facility in Byron, Georgia, that had originally been collected in the field near Gainesville, Florida, and had been in colony for about 10 yr. The insects were reared at about 25°C, 70% RH, on immature apples from Washington state that were picked when about 3 cm in diameter. Larvae emerging from the apples were placed on sterilized potting soil until adult emergence.

Ten 2-week-old adults of each sex were placed on 200-300 g of immature fruits listed in Table 1 for 3 days. Observations on feeding, oviposition, and mortality upon removal from the fruit were made. The fruits were held at about 25°C, 70% RH for 2 weeks after which they were examined for feeding damage and larvae. Feeding larvae were allowed to continue development. When they emerged from the fruit, larvae were placed on potting soil for pupation and adult emergence. Adults that developed on loquat were placed on immature apples for 3 days to see if the next generation would reproduce. Analysis of variance and Tukey's Multiple Comparison Test were done with Prism 3.0 (GraphPad Software, San Diego, CA).

Superficial feeding, but no oviposition, was observed on the stem wound of all fruit species. Of course, this type of feeding would not occur while the fruit was on the plant. Strong feeding occurred to apple, plum, and peach, hosts often requiring plum curculio control. Strong feeding was observed on the only other rosaceous fruit studied, loquat, and on passion fruit, a Passifloraceae (Table 1). Oviposition, assessed by opening suspected oviposition scars, was not observed on passion fruit. Because passion fruit suffered so much feeding, additional tests with 80 more adult plum curculios were conducted. The fruits in these additional tests suffered similar feeding damage, but no oviposition or larval damage was observed.

A considerable amount of feeding occurred on mango and Barbados cherry (Table 1), but no oviposition or larval damage was observed. Lesser amounts of feeding occurred on 11 other fruit species, while none (except for stem-end) was found on seven. No eggs were found on any of these fruits. There were no statistically significant differences in adult mortality upon removal from the hosts.

Larvae grew and developed to adults on all of the rosaceous fruits. The pest-host relationship between plum curculio and loquat is not well known; we know of no other studies of loquat as a

TABLE 1. FRUITS OFFERED TO PLUM CURCULIO AND MORTALITY OF ADULTS UPON REMOVAL

Fruit (Family)	Common name	Source[a] no. replicates	Fruit Weight g (SEM)	Fruit Diameter cm (SEM)	Feeding[b]	Mortality[c] upon removal % (SEM)
Mangifera indica L. (Anacardiaceae)	Mango	F3	144 (33)	5.6 (0.7)	much	1.7 (1.7)
Spondias purpurea L. (Anacardiaceae)	Red mombin	F3	8.3 (1.1)	2.2 (0.12)	some	3.3 (1.7)
Annona squamosa L (Annonaceae)	Sugar apple	F3	39.6 (21.8)	4.0 (0.9)	none	1.7 (1.7)
Cordia boissieri A. DC. (Boraginaceae)	Texas olive	T4	5.0 (3.1)	2.1 (0.39)	some	0
Ehretia anacua (T.&B.) Johnst. (Boraginaceae)	Sandpaper tree	T2	0.4 (0.09)	0.86 (0.1)	little	0
Carica papaya L. (Caricaceae)	Papaya	T4	31.3 (18.8)	3.6 (0.6)	v. little	7.5 (3.2)
Diospyros digyna Jacq. (Ebanaceae)	Black sapote	F3	60 (42)	4.9 (1.6)	none	0
Persea americana Miller (Lauraceae)	Avocado	F4	52.2 (32.7)	4.0 (0.95)	none	2.5 (1.4)
Malpighia glabra L. (Malpighiaceae)	Barbados cherry	F4	1.2 (0.67)	1.3 (0.27)	much	0
Eugenia uniflora L. (Myrtaceae)	Surinam cherry	F3	0.66 (0.27)	1.1 (0.17)	some	3.3 (3.3)
Psidium guajava L. (Myrtaceae)	Guava	T4	22.9 (5.0)	3.2 (0.2)	v. little	5.0 (2.0)
Syzygium jambos Alston. (Myrtaceae)	Rose apple	T3	9.6 (2.7)	2.6 (0.3)	v. little	5.0 (5.0)
Averrhoa carambola L. (Oxalidaceae)	Carambola	F3	6.8 (6.3)	2.4 (1.1)	none	2.5 (2.5)
Livistona chinensis (Jacq.) R. Br. ex Mart. (Palmae)	Chinese fan palm	T4	1.6 (0.3)	1.2 (0.09)	v. little	2.5 (1.4)
Passiflora edulis Sims. (Passifloraceae)	Passion fruit	T4	46.5 (9.8)	4.8 (0.3)	strong	5.0 (2.0)
Eriobotrya japonica Lindl. (Rosaceae)	Loquat	T4	8.9 (1.9)	2.4 (0.14)	strong	1.3 (1.3)
Malus domestica Borkhausen (Rosaceae)	Apple	W4	12.9 (1.9)	2.8 (0.3)	strong	2.5 (1.4)
Prunus domestica L. (Rosaceae)	Plum	V4	9.6 (1.9)	2.3 (0.2)	strong	13.8 (2.4)
P. persica (L.) Batch (Rosaceae)	Peach	V2	20.5 (4.4)	3.1 (0.2)	strong	10.0 (0)
Casimiroa edulis Llave & Lex. (Rutaceae)	White sapote	F3	31.2 (11.3)	3.7 (0.5)	some	3.3 (1.7)
Citrus sinensis (L.) Osbeck (Rutaceae)	Orange	T4	34.5 (15.3)	4.0 (0.6)	none	11.3 (9.7)
Litchi chinensis Sonn. (Sapindaceae)	Lychee	F3	10.9 (2.5)	2.5 (0.15)	none	3.3 (1.7)
Pouteria campechiana Baehni. (Sapotaceae)	Canistel	F3	171 (63)	6.8 (1.0)	some	3.3 (1.7)
P. sapota (Jacq.) HE Moore & Stearn (Sapotaceae)	Mamey sapote	F3	202 (285)	5.7 (2.8)	none	6.7 (3.3)
Solanum pseudocapsicum L. (Solanaceae)	Jerusalem cherry	T4	0.36 (0.09)	0.9 (0.08)	v. little	5.0 (5.0)

[a]Source: F, USDA-ARS Subtropical Horticulture Research Station, Miami, Fla.; T, USDA-ARS Subtropical Agricultural research Center, Weslaco, Tex., except for loquat, which was from nearby McAllen, Tex.; V, USDA-ARS Appalachian Fruit Research Station, Kearneysville, W. Vir.; W, USDA-ARS Yakima Agricultural Research Laboratory, Wapato, Wash.

[b]Feeding: descriptive classes in descending order of damage: strong (typical damage to known good hosts), much (considerable damage, but noticeably less than strong), some (noticeable, but limited, feeding on a few of the fruits), little (damage less than some), v. little (small and very few feeding holes on peel), none (only some superficial feeding on stem end wound).

[c]Mortality: $P = 0.30$, $F = 1.179$, $df = 24, 59$ (no significant differences).

TABLE 2. MEAN NUMBERS AND WEIGHTS OF LAST INSTAR LARVAE EMERGING FROM HOSTS OF PLUM CURCULIO INFESTED BY 10 PAIRS FOR 3 DAYS.

Host	Larvae emerged (SEM)	Larval weight mg (SEM)
Apple	10.5 (1.7)	18.4 (0.4) a
Peach	11.5 (3.5)	16.3 (0.3) ab
Loquat	4.5 (0.6)	14.0 (0.7) bc
Apple[a]	—	14.0 (1.0) bc
Plum	8.0 (2.2)	12.5 (0.6) c

Means in the same columns are not different ($P = 0.05$) if followed by the same letter (Tukey's Multiple Comparison Test). No significant differences for numbers of larvae emerged.

[a]Parent generation reared on loquat; different number of adults used; no direct comparison of larvae emerged made.

host of plum curculio. Loquat is indigenous to southeastern China and possibly southern Japan; today it is found throughout the tropics and subtropics. In the continental U.S., loquat fruits as far north as about the geographical limit of plum curculio in Florida and in southern Texas and California. The tree will grow, but not set fruit, farther north into the range of southern strain plum curculio. The occurrence of this fruit and other rosaceous fruits in the highland tropics and subtropics shows that potential hosts of plum curculio exist in these regions. Maier (1990) found that northern strain plum curculio infestations were greater in exotic than native hosts. There were no significant differences in numbers of larvae produced per 10 pairs of adult plum curculio per 3 days among the 4 rosaceous hosts ($F = 2.7$, $df = 3, 10$, $P = 0.11$). Larvae reared on apple and peach were significantly heavier than those reared on loquat and plum ($F = 13.9$, $df = 4, 13$, $P = 0.0001$) (Table 2). Mean larval weight for plum curculio reared on apple from adults reared on loquat (14 g) was significantly lower than when both generations were reared on apple (18.4 g). Rapid drying of the immature loquats and plums may have contributed to less favorable conditions for larval development than experienced with immature apples and peaches, which remained moist throughout larval development. But there is no explanation for why larvae reared on apples from adults that were reared on loquats weighed significantly less.

Wayne Montgomery, USDA-ARS, Miami, Florida provided many of the fruits. Tracy Leskey, USDA-ARS, Kearneysville, W. Virginia provided plum and peach. Sandra Ramos and Miguel Diaz, USDA-ARS, Weslaco are acknowledged for their technical help. James Everitt, USDA-ARS, Weslaco identified Jerusalem cherry. Leskey and Diana Alston, Utah State University, Logan, reviewed the manuscript. Comments from two anonymous reviewers are appreciated.

SUMMARY

Southern strain plum curculio, *Conotrachelus nenuphar* (Herbst), was offered 22 immature tropical and subtropical fruits from 16 families to explore its possible host range outside of temperate regions. It fed to varying degrees on the different fruits, but oviposited and completed development only on rosaceous fruits (apple, plum, and peach), including loquat, *Eriobotrya japonica* Lindl.

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REGULATORY ENTOMOLOGY AND BIOLOGICAL CONTROL: A TRIBUTE TO REECE SAILER

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Dr. Reece I. Sailer joined the faculty of the Entomology and Nematology Department, University of Florida, Institute of Food and Agricultural Sciences in early 1973, an opportune time to conduct biological control research (Fig. 1). He entered arguably the nations largest entomological community with a well-developed research and regulatory infrastructure. His research in insect biological control required taxonomists to provide authoritative identifications and an associated library, well-curated collections of specimens identified to species, and a secure building with limited access to contain exotic arthropods and arthropod pathogens. I committed my career to the creation of this kind of infrastructure for Florida and, with the help of a dedicated staff, obtained and managed many of the resources that supported Dr. Sailer. This work began 49 years ago, in July 1953, when I left the Entomology Department of the University of Florida and accepted a position as an Entomologist in the Entomology Bureau of the State Plant Board (SPB). I had been teaching biological control and apiculture, and was interested in insect taxonomy. Ed Ayers, the recently appointed Plant Commissioner, directed me to build an Entomology Bureau with an extensive insect collection and a regulatory capability second to no other state. Although the Bureau was not conducting biological control programs at that time, I was inspired by this opportunity to build for the future.

Fig. 1. Dr. Reece I. Sailer.

The Florida State Collection of Arthropods (FSCA) had less than 30,000 specimens prior to 1953, most of them poorly pinned and without labels. Today, it is one of the best collections in North America with more than eight million specimens well-pinned, labeled and identified to species. The professionally-curated collection also contains partially identified specimens, 344,000 in vials of alcohol and 340,000 mounted on slides. Of the specimens on slides, 100,000 are mites. There are 30,000 bulk samples in alcohol that contain millions of specimens collected across much of the world to be sorted, identified, labeled and added to the collection. Now the fifth largest arthropod collection in the U.S., it is curated by eight full-time taxonomists, one quarantine entomologist, six technicians, and five assistants. Dr. Howard Weems developed the supporting FSCA Research Associates program that has grown to more than 300 members. The taxonomic library is one of the most complete in North American, containing both American and European journals. These FSCA resources at the Florida Department of Agriculture and Consumer Services (DACS), Division of Plant Industry (DPI) are available to the staff, University of Florida faculty and students, and cooperators.

In 1870, Germany was the first country to pass a quarantine law, perhaps due in part to the threat posed by the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). This pest was first described by Thomas Say as an obscure chrysomelid found feeding on a wild solanaceous plant known as buffalo bur or sand bur that grows along the eastern slopes of the Rocky Mountains from Canada to Texas (Say 1824). For 30 years it continued to be of no economic importance until the pioneer settlers brought Irish potatoes into the area. The beetle quickly accepted the potato as a new source of food and became known as the Colorado potato beetle. It swept steadily eastward and arrived at the Atlantic Coast in 1874. This event did not go unnoticed in Germany but, with the help of man, the beetle found its way into Europe and eventually Germany in spite of the quarantine law.

The Florida Plant Act was passed on April 30, 1915 (Yonge 1915), soon after the U.S. enacted the Federal Plant Quarantine Act in 1912 and California became the first state to have regulatory authority, also in 1912. Regulations were re-

quired to control the spread of citrus canker that was found in Jefferson County on September 30, 1912. Some citrus was grown in the panhandle of Florida at that time, particularly Satsuma oranges. The Florida Department of Port and Railway Inspection of the SPB was created in June 1915 to inspect incoming ships and trains. The SPB was in charge of port inspections in Florida until 1958. It was administered by the Board of Control of the University of Florida and furnished office space on the fifth and sixth floors of the Seagle Building until moving to the Doyle Conner Building in 1967 (Fig. 2). The SPB became the DPI, one of the 11 divisions of DACS in 1960. The U.S. Department of Agriculture (USDA) also had their personnel at ports in Florida and, in 1958, accepted full responsibility for 100% inspection of all incoming cargo. Since 1960, the percentage of inspected cargo has steadily decreased to only 2-3% today. This decline was due to a large increase in the volume of cargo entering U.S. ports, including plants, other agricultural products, and passenger baggage.

Prior to the Florida Plant Act, there was no regulatory authority to control non-indigenous species in Florida, introduced or adventive, including biological control agents. Comprehensive records were not kept but it is estimated that about 35 species of parasites or predators were released in Florida from 1899 to 1964, based on information from the SPB, University of Florida, and USDA. The first was the vedalia beetle, *Rodolia cardinalis* (Mulsant), introduced into Florida from California in 1899 to control the cottony cushion scale, *Icerya purchasi* Maskell (Berger 1915). The vedalia beetle had been imported previously from Australia into California where it had become a very successful classical biological control agent. The SPB maintained a colony of the vedalia beetle until the mid 1930s and sold them to citrus growers and nurserymen at 10 beetles per dollar. Cottony cushion scale is no longer a pest in Florida probably because the vedalia beetle is still present and suppressing it to very low population levels. This classic example of biological control caught my attention as a student and I have been interested in the field ever since.

In addition to intentionally introducing insects for whatever purpose, some pests have arrived accidentally on agricultural products. The gypsy moth, *Lymantria dispar* (L.), was imported into the Northeast from Europe in about 1869 to improve silk production. Egg masses are routinely found in Florida on campers and travel trailers that arrive in the fall from infested areas. However, due to Florida's warm climate, this pest does not become established. The European corn borer, *Ostrinia nubilalis* (Hubner), was accidentally introduced in about 1908 on broomcorn from Italy or Hungary. By 1938, it had spread over practically all of New England. It reached the Florida Panhandle in 1975 when it was found in seven counties by DPI inspectors. The Japanese beetle, *Popillia japonica* Newman, was introduced into

Fig. 2. The Seagle Building (A) and Doyle Conner Building (B) in Gainesville, Florida successively housed the State Plant Board.

New Jersey on roots of nursery stock in about 1916 from Japan and spread south into Georgia in the late 1980s. It has been found in Florida as a hitchhiker but has not become established.

Among the more recent invasive pests is the sugarcane rootstalk weevil, *Diaprepes abbreviatus* (L.), first found in the Apopka area (Woodruff 1964). It is a native of the West Indies with many host plants, including ornamental nursery stock. Initially, only one specimen was discovered on a nursery plant, but an infestation was subsequently detected in a nearby citrus grove (Poucher 1968). The adults feed and lay eggs on the leaves of more than 200 hosts and larvae cause serious damage by feeding on roots. Larvae are difficult to control with insecticides after the first instar, even when chlordane, aldrin and dieldrin were available. A hymenopteran parasite, *Quadrastichus haitiensis* (Gahan) was released in the Apopka area but it was not effective because it could not overwinter. More recently, Dr. Jorge Peña reported that the egg parasites, *Q. haitiensis*, *Ceratogramma etiennei* Delvare and *Aprostocetus vaquitarum* (Wolcott), have been released and recovered in the southern part of the state (Peña 2002). The sugarcane root weevil has spread through 19 counties infesting more than 150,000 acres. The USDA's research showed that a kaolin-based particle film sprayed on the leaves reduced feeding damage by 68-84%. The film also prevents egg masses from sticking to the leaves, causing them to drop to the ground and either desiccate or be consumed by predators.

The Mediterranean fruit fly, *Ceratitis capitata* (Wied.), invaded Florida in 1929. As a requirement to ship fruit, growers had to keep the groves clean. Small boys were hired to collect fruit that dropped from trees and bury it three feet deep. At eight-years-old, I was paid 10 cents per hour to accomplish this task. The bottom limbs of citrus trees were not pruned in those days and it was difficult to crawl under them. As an adult, I have been involved in every medfly eradication program in Florida through 1990. The medfly has been trapped in Florida another 16 times. Extensive trapping followed single fly catches and aerial bait sprays were applied if additional flies were caught. The medfly was eradicated from Florida each time two or more flies were found. DNA studies of this fly have helped to determine pathways of introduction and prevent future infestations. Eradication is very expensive and the public is getting less tolerant of bait sprays. As an alternative, sterile male flies have been released in Hillsborough, Manatee, Sarasota, and Miami-Dade Counties since May 1998. Florida has been medfly free since October 1998 and, hopefully, sterile male releases will prevent infestations in the future.

The Caribbean fruit fly, *Anastrepha suspensa* (Loew), was discovered at Key West in 1935 and 175 flies were trapped during the next two years. During this period, only 20 flies were trapped in Dade, Broward, Palm Beach and Lee Counties. This species was not detected again in Florida until April 23, 1965 at Miami Springs, and it subsequently infested Surinam cherries in the same area. Federal personnel who worked in Puerto Rico thought that the caribfly was only a pest of ripe grapefruit, so it could not overwinter in Florida. Malathion (LV 95%) applied at the rate of 2 oz per acre and 5 oz (25% WP) plus 1 pint of SIB No. 7 bait per acre were tested on 200 acre blocks at Miami in 1965. Both sprays provided control sufficient for eradication of the Caribbean fruit fly. The Florida Legislature offered $1 million for an eradication program; however, it was deemed unnecessary based on information provided by the USDA, APHIS. Within a year, the caribfly spread to 30 counties and we have had to learn to live with it.

The citrus blackfly, *Aleurocanthus woglumi* Ashby, first detected and eradicated in 1935 at Key West, was found a second time on January 28, 1976 in Broward County. A survey indicated that Dade and Palm Beach Counties were also infested. DPI and APHIS attempted to eradicate the citrus blackfly using malathion at 12 oz per 100 gallons of water and later increased the rate to 20 oz per 100 gallons applied every 14-21 days. However, the treated areas became reinfested between applications, so the insecticide program was discontinued and two parasites were introduced from Mexico, *Encarsia perplexa* (Silvestri) and *Amitus hesperidum* (Silvestri). These natural enemies were controlling citrus blackfly in Mexico to a level below the economic threshold (Browning & Stimac 1994). At first, the Florida citrus growers did not believe that the parasites would control the blackfly and wanted it to be eradicated but Dr. Sailer monitored the parasite releases, observed the numbers of parasitized blackflies, and predicted that the wasps would provide excellent control in Florida as they had in Mexico. Blackfly infestations are occasionally encountered today and the growers are usually asked to not use insecticides on their citrus to give the parasites a chance to multiply. A similar situation occurred with classical biological control of Florida red scale, *Chrysomphalus aonidum* L., by the parasite, *Aphytis holoxanthus* De Bach.

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton, was first found in Florida in May 1993 and infested most of the commercial citrus plantings in the state within six months. The larva of this moth feeds on young leaves and forms serpentine mines. The citrus producers sponsored a classical biological control program in 1994 to import, rear and release the Asian parasite, *Ageniaspis citricola* Loginovskaya, even though the CLM was already serving as a host for as many 13 species of native parasitoids (Browning & Pena 1995). The CLM populations declined significantly

in 1995, ostensibly due to *A. citricola* causing 60% control in 28 counties. Ants and ladybeetle larvae tear open mines and remove CLM larvae. Lacewing larvae, spiders and hemipteran predators simply pierce the mine and drain the body fluids from the larvae. A study of CLM in south Florida lime groves indicated that predators are the most important cause of mortality, killing about 50 percent of the population in most years (Amalin et al. 2001). It is quite likely that certain predators increase in abundance, especially those for which CLM represent a suitable food.

The brown citrus aphid (BCA), *Toxoptera citricida* (Kirkaldy), was discovered in south Florida in 1995 and quickly spread to nearly all commercial citrus growing areas. By 1997, major infestations of BCA were found throughout the state and the trees were covered with sooty mold. BCA is very efficient in the transmission of citrus tristeza virus (CTV). A parasite, *Lysiphlebia japonica*, was imported from Japan, mass reared and released at over 30 sites throughout the state but it apparently has failed to establish. Regardless, BCA populations have continued to decline as biological control improves due to a combination of generalist insect predators, primarily certain species of lady beetles and hover flies. Localized outbreaks may still occur, however, if biological control is disrupted by grove mismanagement (Michaud & Browning 1999). CTV transmission remains a problem, however, and it is doubtful that any natural enemies, whether native or exotic, will be able to reduce the incidence of CTV to the pre-BCA levels.

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (ACP), was discovered in Florida by Dr. Susan Halbert and Ellen Tannehill, a DPI plant inspector, on June 2, 1998 during a routine survey of citrus for CTV. ACP is the primary vector of greening disease in Asia but, fortunately, this disease has not been detected in Florida, although trees are inspected continuously for its presence. Two parasites, *Tamarixia radiata* (Waterston) and *Diaphorencyrtus aligarhensis* (Shafee, Alan and Agaral), have been released at multiple sites in citrus growing areas of Florida (Hoy & Nguyan 2001). A wide range of generalist predators capable of developing on ACP has been identified, including lacewings, hover flies, ladybeetles and spiders. Most notable is the native ladybeetle, *Olla v-nigrum* (Mulsant), that has also been successfully introduced into Asia for biological control of *Heteropsylla cubana* Crawford, a psyllid of Caribbean origin (Michaud 2001).

Prior to 1965, no state agency regulated the movement of parasites and predators into and within the State of Florida. The USDA, APHIS issued a Plant Protection Permit (Form PPQ 526) for shipments of parasites or predators as a courtesy to expedite their entry into the U.S. or movement from state to state. Therefore, without clear authority but at the urging of the researchers in Florida who were interested in getting clearance for testing parasites and predators, I established the Arthropod Introduction Committee (Denmark & Porter 1973). Later, "Arthropod Pathogen" was added to the committee's title. The committee included representatives from the various organizations with an entomological interest: Florida Division of Health and Rehabilitative Services, DPI, Bureau of Entomology (now under DACS) and Division of Animal Industry; U. S. Public Health Services (USPHS), Veterinarian, Communicable Disease Center (Atlanta, Georgia); Florida Game and Fresh Water Fish Commission (FGFWFC), and the UF, Entomology and Nematology Department. When a request to import or release a natural enemy was received, I sent it to the committee members for their recommendations. Following committee approval, the request was forwarded to the DPI Director, Halwin Jones, for his concurrence. Although he was not particularly interested in the committee at that time, he agreed to sign the PPQ Form 526 and forward it to Beltsville, Maryland for final approval by USDA, APHIS. If approved, APHIS issued a shipping permit to the requesting individual and sent a copy to DPI. Voucher specimens of species being tested for safety and released into the environment were housed in the FSCA. If there was any question about the identity of a species released into the environment, it was available for further study. Due to an increased interest in conducting research on parasites and predators, as well as economic pests, Hal Jones decided to make the Arthropod and Arthropod Pathogen Introduction Committee official. He convened a meeting on September 22, 1965 in Orlando to formulate an addendum to the Florida Statutes making unauthorized movement of arthropods into and within the state a misdemeanor, and imposing a maximum penalty of one year imprisonment and a $5,000 fine (Denmark 1988).

In 1970, the Florida Legislature passed a bill giving the FGFWFC control over movement of all animal life into and within Florida. It was decided that DACS and FGFWFC would cooperate in the regulation of arthropods by means of FGFWFC representation on the Arthropod and Arthropod Pathogen Committee. Evidently it was the intent of the Florida Legislature for FGFWFC to only regulate the movement of vertebrates. The Florida Legislature consequently passed a bill in 1973 granting the DPI control over all plant pests. In 1990, the Florida Legislature passed a bill giving DPI control over pet shops selling spiders, tarantulas, scorpions, cockroaches, walkingsticks, beetles, moths and other exotic arthropods deemed a public nuisance if released into the environment. APHIS did not regulate these arthropods because they were not considered to be plant pests. A box containing specimens of the arthropods from pet

shops was sent to Tallahassee for the legislators to view. It was immediately named the little box of horrors and the bill passed without any question. The DPI also monitors companies selling arthropods for other purposes, particularly biological control, to ensure that the species being sold is the one advertised. To my knowledge, Florida is the only state that identifies commercial species sent for verification every other year. Companies may not be aware that their cultures of small arthropods, such as mites, are contaminated. Actual samples assumed to contain a single species have been found to contain as many as three. This happens because almost no company employs a taxonomist who can identify or separate the species they are selling. Occasionally, for example, scavenger mites decimate the original colony. These problems can cause growers and homeowners to lose faith in biological control, particularly if no or minimal control is realized.

I visited the quarantine facility at Riverside, California in 1966 to formulate plans for a quarantine building in Florida. Plans were drawn and reviewed by colleagues who had experience in working in a containment building. The final plans were included in my budget for the next legislative session and sent to the DPI director for his approval. He thought the idea was good, but that the time was not right. The plans were included in my budget request for the next six years and I was told each year by the Commissioner of Agriculture, Doyle Conner, that my request was 3rd in priority but it never seemed to advance. The budget director in the governor's office was a strong supporter of biological control and, after being discretely informed of the request for a security building, added it into the budget that reached the governor. Such tactics were cause for termination of one's career with the state, if known by one's supervisor. Nevertheless, the first security building in Florida was dedicated in June 1973 at the DPI in Gainesville (Fig. 3). The primary purpose for the security building was to remove parasites and predators from host material, separate and remove any hyperparasites, and test the natural enemies against target pests before requesting permission to release them into the environment. Workspace was provided for USDA, ARS; University of Florida, Entomology and Nematology Department; and DPI scientists to conduct research in biological control. Space in this building was adequate for about 10 years. Plans for a second security building (Fig. 3) were submitted in 1983 and it was dedicated in 1989, after Senator Kirkland was persuaded to put the building request back into the Legislature's budget.

In 1973, Dr. Reece Sailer accepted the position of graduate research professor in the Entomology and Nematology Department at the University of Florida, Gainesville and was assigned space in one of the security laboratories to conduct his research. Dr. Sailer had previously served as a taxonomist responsible for the identification of true bugs and allied research for the USDA's Insect Identification and Beneficial Insect Introduction Branch of the Entomology Research Division. He was partially interested in the southern green stinkbug, *Nezara viridula* (L.), complex. He had numerous field assignments, including a study of the effects of DDT on forest fauna, and biological and ecological investigations on mosquitoes and other biting flies in Alaska. He became assistant chief of the Branch in 1960 and moved to Paris, France to serve as director of the European Parasite Laboratory. He returned to Beltsville, Maryland in 1966 and was appointed Branch Chief. Just before retiring from the USDA, he served as Chairman of the Insect Identification and Beneficial Insect Introduction Institute.

While at the University of Florida, he demonstrated that inoculative releases of the imported parasite, *Pediobius foveolatus* (Crawford), could provide effective control of the Mexican bean bee-

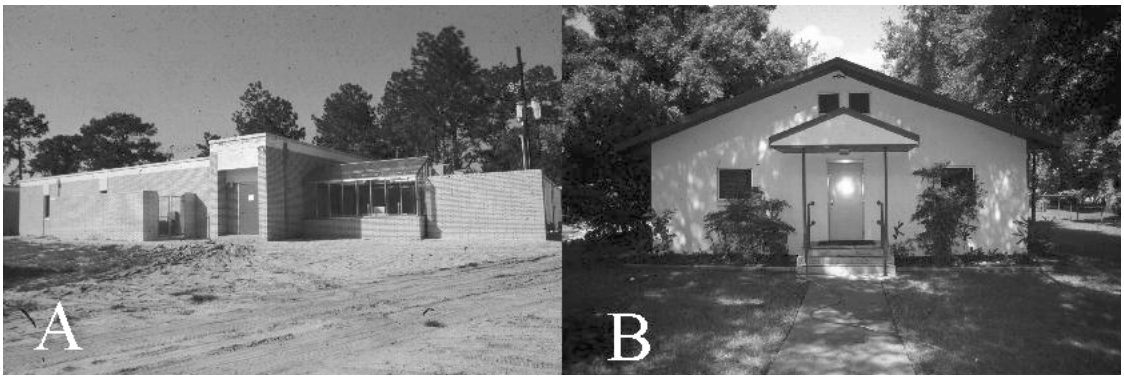

Fig. 3. First Security Building (A) and Second Security Building (B) at the Florida DACS Division of Plant Industry in Gainesville.

tle, *Epilachna varivestis* Mulsant. He was so pleased with this success that he promised to eat any beetles that were found in the parasite release areas. His student assistant, Limhuot Nong, searched for the beetles with great enthusiasm and determination, finding 12 at the organic gardens on campus. Being a man of his word, Dr. Sailer reported this find to the Florida Entomology Society at the next annual conference and ate them at the general session. One of his most significant achievements was importation of the parasite, *Encarsia lahorensis* (Howard), that provided excellent control of the citrus whitefly, *Dialeurodes citri* (Ashmead). He also was successful in establishing a South American mole cricket parasite, *Larra bicolor* Fab., in southern Florida. Although Florida still has tea scale, *Fiorinia theae* Green, Dr. Sailer introduced *Aphytis theae* (Cameron) to control it, including an infestation in my camellia garden. I sprayed an insecticide at least twice each year before the releases but *A. theae* completely controlled the tea scale after six months. He made the release more than 20 years ago and my camellias are still free of tea scale. Dr. Sailer's research will have a lasting effect on Florida's ecology and economy, and we are forever grateful for his contributions.

Acknowledgments

I thank the society for inviting me to present the seventh Pioneer Lecture. It has been a privilege to honor Dr. Reece I. Sailer. I also thank Dr. N. C. Leppla for nominating me to be a Florida Entomological Society Pioneer Lecturer, his help with writing my paper, and assistance in preparing the presentation. Drs. G. A. Evans, S. Halbert and J. P. Michaud graciously provided information and suggestions for the lecture. D. J. Sonke assisted with electronic editing.

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