ON RESEARCH AND ENTOMOLOGICAL EDUCATION II: A CONDITIONAL MATING STRATEGY AND RESOURCE-SUSTAINED LEK(?) IN A CLASSROOM FIREFLY (COLEOPTERA: LAMPYRIDAE; *PHOTINUS*)

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ABSTRACT

The Jamaican firefly *Photinus pallens* (Fabricius) offers many opportunities and advantages for students to study insect biology in the field, and do research in taxonomy and behavioral ecology; it is one of my four top choices for teaching. The binomen may hide a complex of closely related species and an interesting taxonomic problem. The *P. pallens* population I observed gathers in sedentary, flower-associated swarms which apparently are sustained by the flowers. Males and females remained together on the flowers for several hours before overt sexual activity began, and then pairs coupled quickly and without combat or display. Males occasionally joined and left the swarm, some flying and flashing over an adjacent field in a manner typical of North American *Photinus* species.

Key Words: Lampyridae, *Photinus*, mating behavior, ecology

RESUMEN

La luciérnaga jamaiquina *Photinus pallens* (Fabricius) brinda muchas oportunidades y ventajas a estudiantes para el estudio de la biología de los insectos en el campo y para la investigación sobre taxonomía y también sobre ecología del comportamiento; es una de las cuatro opciones principales elegidas para mi enseñanza. Este nombre binomial puede que incluya un complejo de especies cercanamente relacionadas, que es un problema taxonómico interesante. La población de *P. pallens* que observé se reune en grupos sedentarios asociados con flores los cuales son aparentemente mantenidos por dichas flores. Machos y hembras permanecieron juntos sobre las flores por varias horas antes de que evidente actividad sexual comenzara, y luego las parejas se aparearon rápidamente sin combate ni exhibición. Los machos ocasionalmente se juntaron y abandonaron al grupo, algunos volando y alumbrando sobre un campo contiguo en una forma típica de las especies norteamericanas de *Photinus*.

As in days agone, I take certain truths to be self evident: 1) that the connection between academic research and teaching is that professors who do research maintain their intellectual interest in scholarship and infect their students with a passion and love for a lifetime pursuit of knowledge; 2) that students properly taught become living repositories of this civilizing Ideal of western culture; and 3) that a true academician understands the expression "publish or perish" to mean that he publishes to give evidence that he has not mentally perished, and thus failed in his unique and special responsibilities to his students and civilization. It is in this context that I introduce fireflies that I have met, and suggest research that students can conduct, through Letters similar to those I use as substitutes for lectures in my firefly courses. The one offered here follows through on an observation that I made in last year's symposium (Lloyd 1997), that insect taxon-

omists in particular have a wealth of anecdotes and observations, and even "thumbnail" studies, that can be useful to students and phenomenon-oriented biologists—in keeping with John Sivinski's original introduction to this symposium series. This sketch concerns the quest for an explanation for the long-puzzling congregations of a Jamaican firefly, and for an evolutionary connection between the mating behavior of this firefly and that of its mostly prosaic North American congeners.

One of the puzzles that confronted early fireflyers was the significance of the huge swarms of sedentary fireflies that were reported to occur in some exotic places in the world such as southeast Asia and Jamaica. Once pioneer Frank McDermott had discovered how the flash signal system operates in North American fireflies (McDermott 1917, see Lloyd 1990), sexual attraction in fireflies was understood to be a one-on-one operation. (Recall that in general, the male firefly searches throughout his habitat/site while flashing his species-specific signal, and when a female of his species sees, from her perch below, the appropriate signal [flash pattern] she flashes an answer, and the two maintain a pattern-answer dialogue until he reaches her.) Thus, while the huge gatherings and displays of *Pteroptyx* fireflies long reported to occur along tidal rivers and in mangrove swamps in southeast Asia made little sense, the swarming of a *Photinus* firefly in Jamaica was probably even more inscrutable—should not a *Photinus* species, no matter where it occurred, follow the same general signaling routine and mating protocol? What were the Jamaican *Photinus* doing? Specifically, if their gatherings actually were mating swarms, how did such a signaling system work, with such visual cacophony and all?; and, what was the evolutionary connection between such behavior and the general pattern observed in *Photinus* species in North America?

After the 1985 FES meeting in Jamaica I had a chance to spend a week in the field with this enigmatic firefly, and make observations on swarms along the Rio Grande River, between the Blue Mountains and the John Crow Mountains (Fig. 1). There were many gatherings in the trees and fallow fields along the river and the road that followed it up toward the highlands. The following Letter outlines key observations, provides tentative answers for the two basic questions, and suggests a working model for *P. pallen's* mating system. In the future Lesley Ballantyne and I will publish data on morphological comparisons and luminescent emission patterns.

The Internet (electronic) publication of this paper has additional figures as AuthorLink attachments to illustrate the text; these are color slides of the study region and site, and various firefly behaviors. These are cited in text here by their number as ALR figures. For example, the figure citation in the preceeding paragraph should read (Fig. 1; ALR 1998, fig. 1) because the first AuthorLink illustration is a view of the Rio Grande River and the road paralleling it. Legends for AuthorLink figures are included here in this printed version in the End Notes section. These copyrighted illustrations may be used freely with this citation: J. Lloyd, Univ. of Florida.

LETTER 23
THE UNIQUE MATING BIOLOGY OF A CONGREGATING FLOWER-LOVING FIREFLY

Dear Fireflyers, the Jamaican firefly *Photinus pallens* (Fabricius) is one of the most interesting and curiously different species that you may ever see, and it is the best reason for going to Jamaica that I can think of. What sets *P. pallens* apart is that its sexual behavior is very different from anything yet found in any species, in Jamaica or any place elsewhere in the Americas. Recalling lessons on the comparative method in biology, this means that this firefly can provide an interesting exploration

into "adaptive" radiation, and may reveal subtle elements in the behavior of other *Photinus* that we now observe, but don't really see.

P. pallens is certainly one of the "World Class Big Four" apropos of firefly research opportunity, and I have several arguments in support of this nomination. 1) It has the richest, most complex mating protocol yet seen in any *Photinus* species, for it apparently involves a conditional sexual strategy, prolonged mate evaluation, conspicuous mutual luminescent displays with "obvious" resource acquisition, and perhaps biparental investment in offspring; 2) this flower-loving firefly with its esoteric sexual charm, commonly occurs in easily accessible, sedentary, and manipulatable swarms—I saw populations numbering in the thousands in fallow fields on the blossoms of the introduced Asian ginger-lily—and the fireflies show varying degrees of site fidelity, which seemingly is related to the number and nutritional value of the arena-plant's blossoms.

3) *P. pallens* is a large and robust firefly, and this makes field observation easier and permits marking for individual recognition, and it also simplifies dissection for analyses of individual mating and nutritional conditions that may be connected with mating behavior; 4) *P. pallens* is widely distributed in time and space, can be found throughout Jamaica and the year (Fig. 1), and it is easily identified by non-coleopterists (Fig. 2); it is readily located in the field and described to residents if help is needed in finding active populations; and 5) *P. pallens* has a taxonomic mystery about it, for though I have referred to it in the singular, as "it", so-called *P. pallens* may actually be an array of closely-related species, incipient species, and sister and cousin populations, diverging through mechanisms of sexual preference while in micro-geographic and/or temporal isolation. On a trip to Jamaica in the 1960s I observed and recorded "a *P. pallens*" with a completely different flash pattern and saw no huge gatherings of them.

My first doctoral student, Ed Farnworth, reviewed what was known about *P. pallens* when he made an extensive study of Jamaican fireflies (1973), and made a number of observations on its behavior, ecology, and distribution. He pointed out the fragmentary nature of current knowledge, alluded to the complexity of the puzz1e, and suggested that what was needed were detailed observations on the behavior of lo-

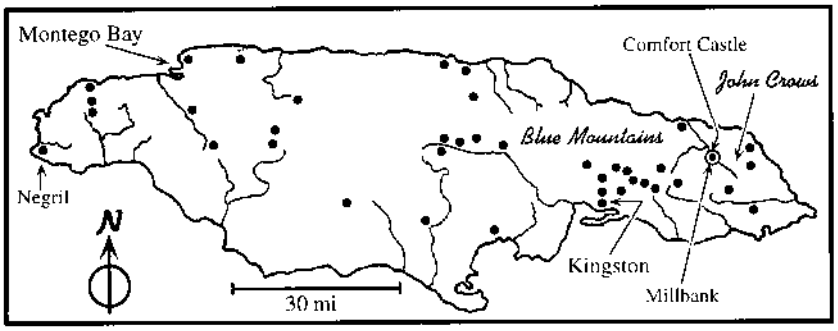


Fig. 1. Known localities of *Photinus pallens* (s.l.) in Jamaica. Elevations range from sea level to 5000 feet; there are records for every month except May. Records are primarily from Farnworth (1973), but also Leng and Mutchler (1922), and personal observation. The circled dot at the eastern end of the island, in the valley of the Rio Grande River between the Blue and John Crow Mountains, is where the observations reported here were made, near the villages of Comfort Castle and Millbank.

cal populations. In August of 1985 the Florida Entomological Society held its annual meeting in Montego Bay, and after the required formalities and speeches, two of us took to the hills, "your present author" with the *P. pallens* puzzle firmly in mind. I found a good population in a ginger lily field between Comfort Castle and Millbank, on the Rio Grande River at an elevation of about 1000 feet—but this jumps ahead in the story.

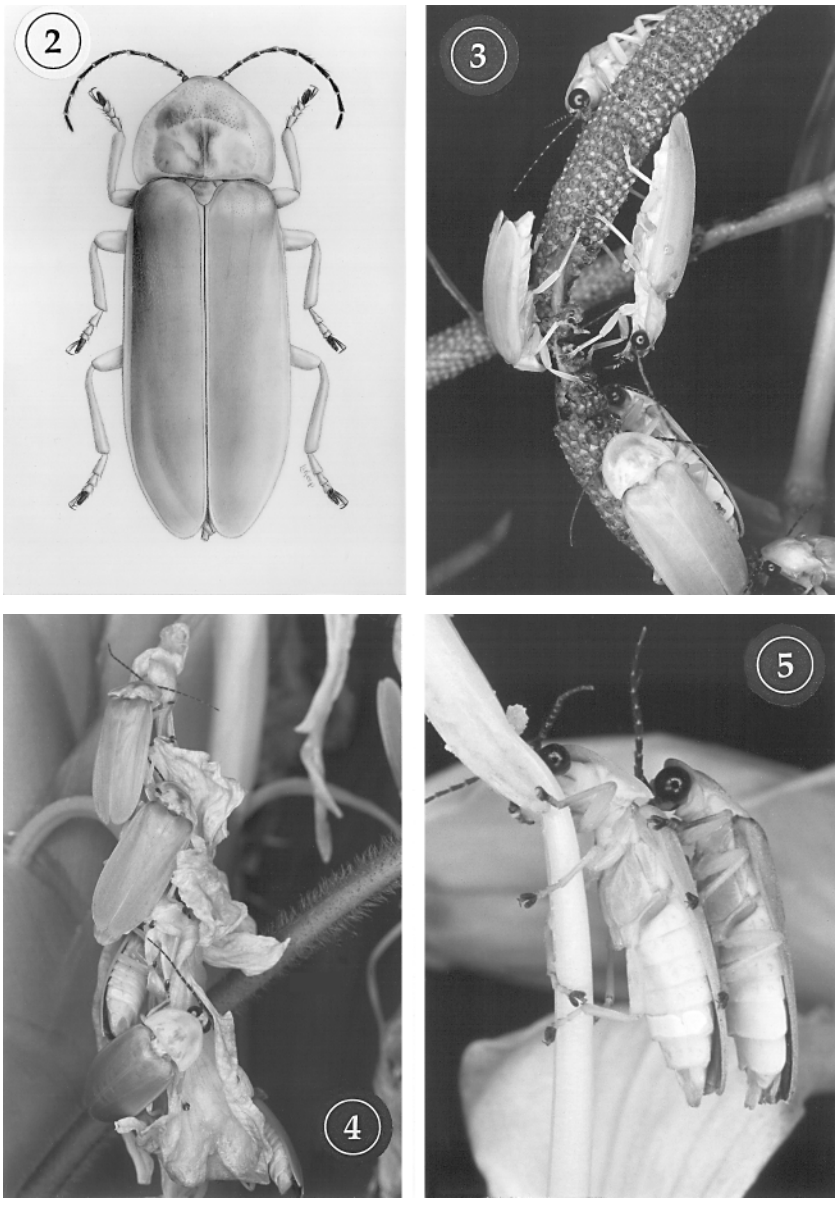
From the literature and my observations it is clear that *P. pallens* congregates on many different plant platforms: almond, rodwood, jointers, water mahoe, thistle, and others, and sometimes in such numbers that the apparition of a swarm can be seen from some dark distance. I saw several in trees along the creek called the Rio Grande River, and could see their light from distances nearly as great as the combined lengths of two football fields. Commonly such gatherings occupied only a portion of a tree, a single branch, or a discrete "patch" in the foliage, but sometimes the entire crown of a tree was occupied with flashers.

Though they occurred on a wide variety of plants and foliage types, walking along stems and on and around the edges of leaves, it was clearly the blossoms of the plants that were of special importance, for on them males and females remained motionless with their mandibles buried in the flower parts. This resulted in an interesting illusion when you looked up through the foliage of a jointers tree; individual flashing lights on the vegetative parts of the tree were separated and in motion, but points of light on the flowers were clustered and fixed along the elongate, curved spikes (Fig. 3; ALR 1998, fig. 2), and appeared as scattered Pleides star constellations in a fluid universe.

With respect to the initial formation of a swarm there can be little doubt that a single flashing individual can seed a gathering. Flashers on a patch of low grass as in a pasture, captives in spiders webs (ALR 1998, figs. 3-5), and even the red-filtered light of a head lamp being used by an entomologist who was digging singing crickets out of their burrows, attracted *P. pallens*. Though swarms formed easily, only those with flowers were sustained for very long. At blossom-rich sites such as jointers trees and ginger lily fields (ALR 1998, fig. 6), many fireflies could be seen at dusk entering the flower-arena from daytime retreats in the grass beneath the plants; "certainly" these were swarmers from the previous night rejoining their swarm.

The Comfort Castle-Millbank region was a mosaic of agricultural and fallow fields, with borders of tall grasses and other herbs and hedgerows of various trees and shrubs. The study-site was a patch of the invasive Asian wildflower, the ginger lily (*Hedychium corium*; ALR 1998, figs. 6-7). Each flower spike had several blossoms, but it was the recently-drooped petals of mid-level flowers, not the top fresh nor the severely withered ones at the bottom of a spike that the fireflies chose to stand on and sink their mandibles into (Fig. 4). Curiously, these petals were of about the same pale color as the fireflies themselves (Fig. 4; ALR 1998, figs. 8-10), and a naturalist's reflex would be that a protective coloration model could be proposed—but, considering the relatively recent introduction of the flower and that the beetles do not remain on the flowers during daylight, this is not likely. The site I finally chose to watch had been a taro field, was about 50 by 75 feet in dimensions, and had been plowed but not disked (harrowed) level, and with its corduroy ridges and ditches it often put me down upon my knees in the dark. Immediately adjacent to this patch was a 5-acre taro field (*Colocasia esculenta*, dasheen), over which *P. pallens* males flew, primarily very late at night, as I will soon describe.

Flashing began at the ginger-patch at dusk, in dark, well-shaded places at the ground beneath the fairly dense canopy of lilies and large leaves. Then, three to five minutes later flashing had moved up onto the flowers. As darkness deepened, a few fireflies flew in and landed on the flowers, and for a few minutes at twilight unlit flyers could be seen by silhouette. During the first hour of flashing there was some move-



Figs. 2-5: 2. Habitus of *Photinus pallens*; a carbon dust drawing by Laura Line. 3. Flowering spikes of a jointers tree, with six *P. pallens* at the blossoms. 4. Five *P. pallens* on wilted petals of a ginger lily. 5. A male *P. pallens* mounted on a female. Note the difference in lantern topography.

ment within the patch, as fireflies glowingly flew from perch to perch, and occasionally even several yards out from the swarm before returning to a flower-spike perch. Contrary to what you might expect, fireflies and other beetles are not necessarily clumsy bunglers when it comes to flying, and they sometimes fly to and from perches in very dim light with considerable precision. On the Pacific island of Espiritu Santo I once saw a large luminescent click beetle fly slowly up to the top wire of a barbed-wire fence, illuminating it with his ventral light-organ, and delicately land on the wire—the equivalent of landing a rowboat in the dark, on a powerline, crosswise, using a kerosene lantern!

Here are a couple of examples of field notes that I made during early firefly flight at swarm trees and the ginger patch: "glow start 2' out, go in and land. See another start and fly few feet to another spot . . . rise with glow, go 1 m up, go 3 m and arc down . . . 4 m high, arc back, fishtail . . . male fly from 1 plant to another, like a projectile trajectory. Like [as though] thrown . . ." During the first hour of activity many fireflies joined the swarm from elsewhere: "watch glower approach firefly tree. 80' out . . . as it got closer it got brighter . . . one in from outside [above], made a corkscrew for 4 cycles, 5" diameter . . . long glow 1/4 bright, 10-15' out, went in to tree and landed . . . occasionally see glower coming down, not know if a recruit coming in or one changing positions . . ." There also were exchanges between the swarm and nearby vegetation: "out from tree, flew around periphery in meandering zigzag course, and landed in an adjacent tree . . . out from tree, glowed and glowed, gradually touched down, 1 m high vegetation 2 m out from tree . . ."

Male flash patterns were of two major types, excluding landing flashes and glows. Males that were perched in swarms emitted fairly short flashes at very irregular intervals. In trains of these flashes, a few or several pulses were given in rapid succession and then the rate slowed and they were emitted at irregular and longer intervals. Whether each male has his own individually unique train, a signature you might say, remains to be seen. Occasionally, a flashing male on a flower walked about with his tail turned down. This resulted in his light being directed forward, and it also dragged his abdomen tip along the substrate; perhaps chemical signals and markers were being deposited?

When *P. pallens* males flew over the nearby taro field they emitted bright flash patterns, consisting of a single flare-like flash. (Such flashes were only uncommonly emitted by perched males or those mounted on females.) Photo-multiplier analysis of these flashes revealed them to be symmetrical in form, and to average about one-quarter second in duration; they were emitted at roughly 3-sec intervals. One would obviously presume that these flashes are comparable to the flash patterns emitted by mate-seeking males of our North American *Photinus* species. Males flying over the taro could be attracted to a penlight by answering their flash patterns with a quarter second flash immediately after their flash, which probably approximates their females' responses.

Females in swarms emitted trains of flashes that were visually indistinguishable from those of males. However, photomultiplier-recordings reveal some differences and careful analyses of lengthy, continuous pm-records are needed. There is one curious aspect of male and female flashing that I find especially interesting, and revealing. The sexual difference in light-organ topography of *Photinus* fireflies is well known; the lantern of males occupies two ventral segments and that of females, only a portion of one segment. As expected, when *P. pallens* males emitted flaring flashes they flashed both segments of their light organ brightly. But, when perched and emitting flash trains males emitted light from only one segment of their lantern. And, when this segment was flashed, light sometimes seemed to scintillate across or race around it, and sometimes only the middle section of it was illuminated. In other words, when

perched and flashing in swarms, males and females have similar emission surfaces—and luminous output(?). Thus the loudness of a male's statement is seemingly not of importance to him as he (apparently) competes in each little flower group, nor is his light a competing beacon for the attraction of passing females. The whispered messages of twinkling fireflies on the flowers are a key to the mating system, and it is their meaning that we must seek, to understand *P. pallens* communication and mating system.

From time to time when watching fireflies and stumped for what to do next, as a matter of habit I compulsively quantify; it may help me see and think. I counted stationary points of light in the ginger-patch by slowly scanning across the top of the arena, punching a hand tally counter with my thumb and pointing through (azimuth) space with the index finger of the tallying hand. When I compared scan-samples of flashes with actual beetle counts for several flower-spikes I found that there were 4-5 times more fireflies flashing than I could count from my stand (a 1-ft earthen hummock), which would indicate that sometimes more than 2,000 fireflies could actually have been present in the ginger lily patch!

I made such scan samples of flashing *P. pallens* at various times during several nights (Fig. 6). They began flashing on the flower spikes about 30 minutes after sun-

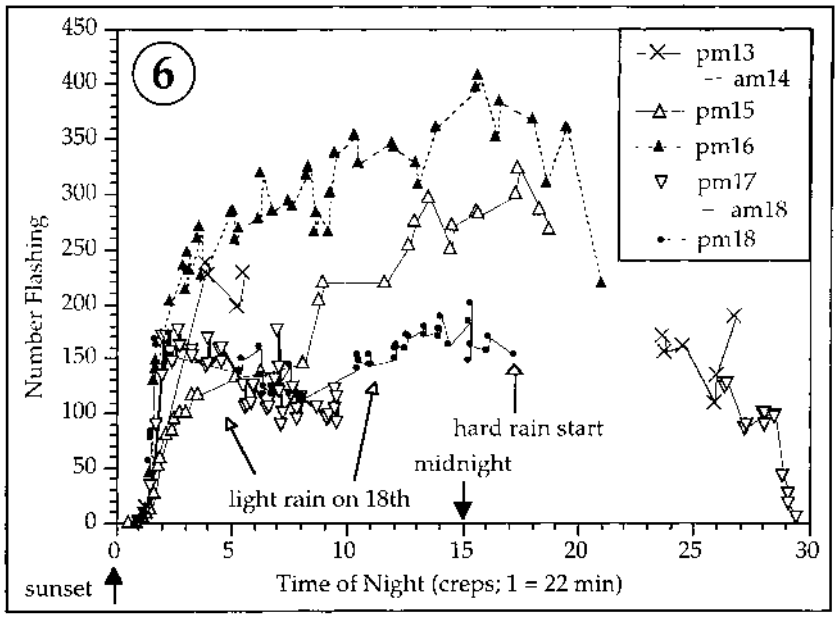


Fig. 6. Scan samples of fireflies flashing in the ginger lily field at various times during five nights. Flashing on the flower spikes began about 25 minutes after sunset, though flashing could be seen in the deep shade at the stem bases a few minutes earlier. An activity peak occurred shortly after midnight (ss + 390 min.) and activity then fell off, ending about 30 minutes before sunrise—about the time birds began singing. The biggest night was on 16 August (pm16). The last two days of observations had the least activity: one followed a warm, dry day, the other, an overcast day with light rain; perhaps the ginger blossoms or the "season" of mating activity in the local *P. pallens* population had reached a peak and was falling off.

set, and the number flashing rose sharply for the next 20+ minutes. Such flashing peaked about midnight (sunset + 360 min.) and completely ended about 25 minutes before sunrise (ss+650). At the end, with the dawn singing of birds, nearly all of the fireflies had left the flowers, most of them apparently having moved down the stems and out of sight, for I saw none in flight.

Up till now though I have alluded to reproduction, and we have come to expect sexual behavior whenever we see adult fireflies flashing, I have not actually mentioned intromission or the physical flowing of sperm and genes. Your suspense should have been mounting, and now it is time for *P. pallens* males to successfully do so—keep in mind that the time of mounting and mating in a local population or swarm could be of considerable significance for recognizing local subpopulations and even presently unrecognized sibling/sister *P. pallens* species.

In another quantification routine, I carefully scrutinized a "trap-line" of (tagged and numbered) individual flower-spikes (ALR 1998, fig. 7) at various times during the night from dusk till dawn seeking recognizable sexual activity. I finally saw it, and it began late, ca 200 minutes after sunset. Before this time of night, though "pairs" were often especially close together, mandibles buried in the same withering petal—even with cuticles touching and standing head-to-head, side-by-side, or lying across each other—nothing conspicuously sexual was noted. In fact, flower-spike samples of "touchy-touchy pairs" had various sex combinations, male/male and male/female and female/female.

Recognizable sexual pairing began when males actually mounted females (Fig. 5), and probed their terminalia with extruded aedeagi (ALR 1998, fig. 8). Males sometimes repeatedly inserted and withdrew the tip or distal portion of their aedeagus (ALR 1998, fig. 9), and at such time both individuals often flashed continuously. Perhaps it is such flashing that is responsible for an increase in overall flashing that seems to occur at about ss + 180 (Fig. 6). Also, at such times males sometimes emitted flare-like flashes, and mounted flaring males could be spotted from some distance in the ginger patch—could such flaring be "desperation arguments" being used on reluctant females? (But this suggestion biases expectations—perhaps it is the males that are the discriminating mate choosers?) The flare-flash has the same form and apparent intensity as the flare-like flash patterns that are emitted over the adjacent taro field. Females easily avoided intromission by bending the tip of their abdomens downward.

Copulation was first observed at ss + 317 minutes (Fig. 7; ALR 1998, figs. 9-10), and sketchy notes and fragmentary observations suggest that pairs may remained attached even until dawn. Soon after connecting, pairs rotated to a tail-to-tail copulation position, and some abandoned the flower petals for adjacent foliage and bracts, with one partner dragging the other backward (ALR 1998, figs. 11-12). At a dawn count, coupled pairs separated abruptly at a touch of their flower or when illuminated by the beam of the headlamp. Males that were rejected apparently did not remain mounted long nor show aggressive behavior toward other males, though I once saw a male briefly butt another that was mounted on a female.

From the aerial traffic I observed it would appear that male *P. pallens* sometimes left their ginger patch flowers and behaved like other ("normal") *Photinus*, seeking females via search over adjacent fields. The temporal appearance of this behavior suggests that there was an intimate and functional relationship between the two activity spaces, between the ginger lily arena and the taro field, and that these two tactics are part of a conditional sexual strategy in this flower lover—conditional in the sense that on condition of mating failure on the flowery platform, or failure to find a swarm, a male ("flashingly") takes to the air to seek a mate afield.

Male *P. pallens* flew over the taro field at altitudes up to 15 feet and their flash patterns could be seen at distances of 75 or more yards. I made a few scan-samples of

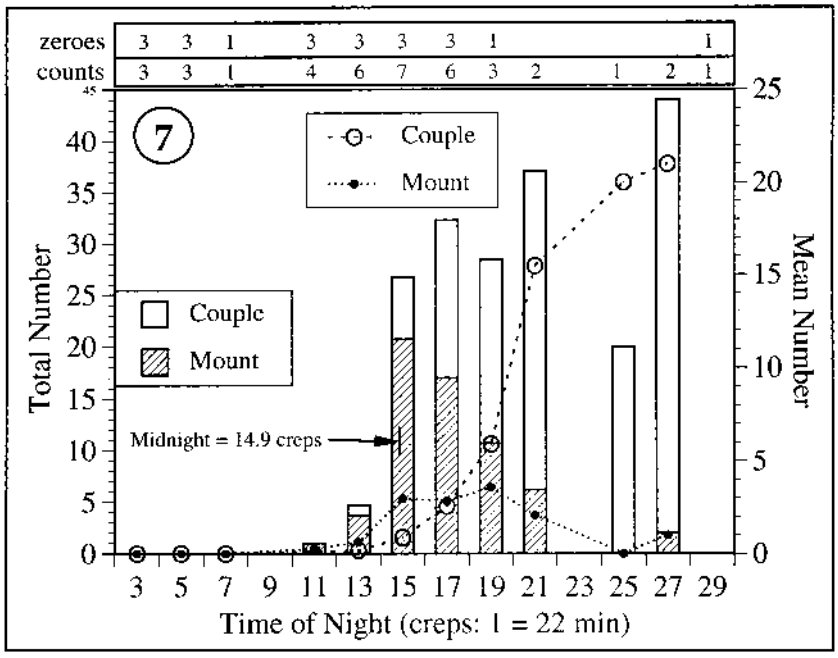


Fig. 7. Number and time of overt sexual activity observed on a sample of ginger lilies. Left Y-axis shows total number (bars); right Y-axis shows mean number (lines and symbols). X-axis shows time of night, with sunset at zero and sunrise at about 30 crep units. Numerals at the top indicate the number (n) the mean was based on, and the number of times no (zero) mounting or copulation was observed in the indicated time bracket.

these airborne flash patterns at various times during five nights. This behavior began about the same time that flashing began in the ginger lily patch, but it remained at a low level until ss + 300, about 30 minutes before midnight, when it increased sharply. My few scattered (in time) samples after ss + 400 indicate that a dramatic, even 15-fold increase may have occurred over the field (Fig. 8). However, it should not necessarily be concluded that such "normal-type" *Photinus* behavior is typically, primarily, or obligately confined to the hours after midnight. I saw many *P. pallens* males afield at other sites along the road early in the evening.—As a bare-bones working notion: perhaps males that eclose in isolation search early in the evening in "typical *Photinus* fashion" until they see the light of a swarm, and males in a swarm may leave it after they determine that their chances of sexual success there are poor. Note that the scan sample data show that the rise in taro search activity occurred at about the time that definitive sexual pairing began on the ginger lilies (compare Figs. 7 and 8).

I began this Letter with two questions about the puzzling flashing and swarming behavior of *P. pallens*: (1) what were the Jamaican *Photinus* doing, and if their gatherings were mating swarms, how did such a mating system work?; and (2) what was the evolutionary connection between such behavior and that of *Photinus* species observed in North America? The flow chart in Fig. 9 is a sketchy working model of how the mating system may operate, and provides an obvious answer to the first ques-

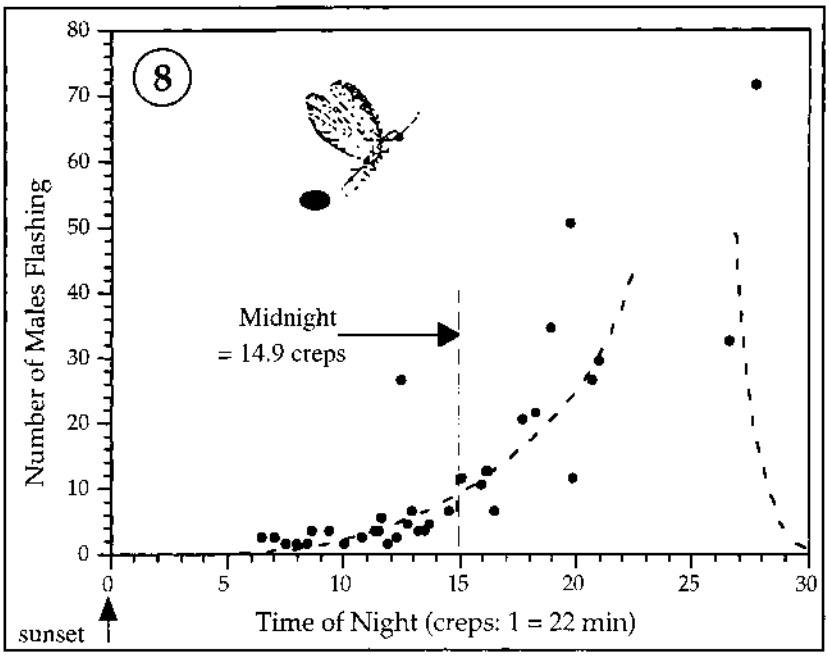


Fig. 8. Scan samples of male *P. pallens* emitting flash patterns over the taro field adjacent to the ginger lily study patch. Males flew up to 5 meters in altitude and emitted their single bright flashes each 2-4 seconds. The beginning of the sharp rise at about 12 creps coincides with the onset of overt sexual activity in the adjacent ginger lily field. Curve segments drawn by eye.

tion—in the flower-borne swarms fireflies find and observe prospective mates and they take on food and water.

The answer to the second question is problematic. We can see that to make a reasonable evolutionary connection, an acceptable historical transition from a typical *Photinus* to the *P. pallens* mating system, we need to insert a stage of lengthy precopulatory association. We might be seeking a *Photinus* species that has prolonged platonic associations at watering or sapping or nectaring holes. Although adult fireflies of various species are occasionally seen at flowers, and captives can be kept alive up to a month by providing them with honey or slices of fresh apple, only the flower-lover *P. pallens* has been found in nature in prolonged association with blossoms. You will need to peek in on the lives and sexual behavior of species that seem to be *P. pallens'* closest relatives, and *pallens* itself (i.e., s.l., in a broad sense) at other Jamaican retreats. Call your travel agent, and when you go, plan ahead to put identifying marks on adults to see, for example, whether sexual associations endure more than one night; and to provide artificial blossoms with various kinds of enriched (e.g., carbohydrate, protein) "juices" to see if they are especially valued; and to see whether molecules of nutrients that males imbibe from flowers wind up in the eggs that their mates lay—could this actually be what the long-delayed copulation and mate choice is all about? Personally, I am most curious about the possibility that the trains of flashes emitted by perched males and females are individualized signatures, because this

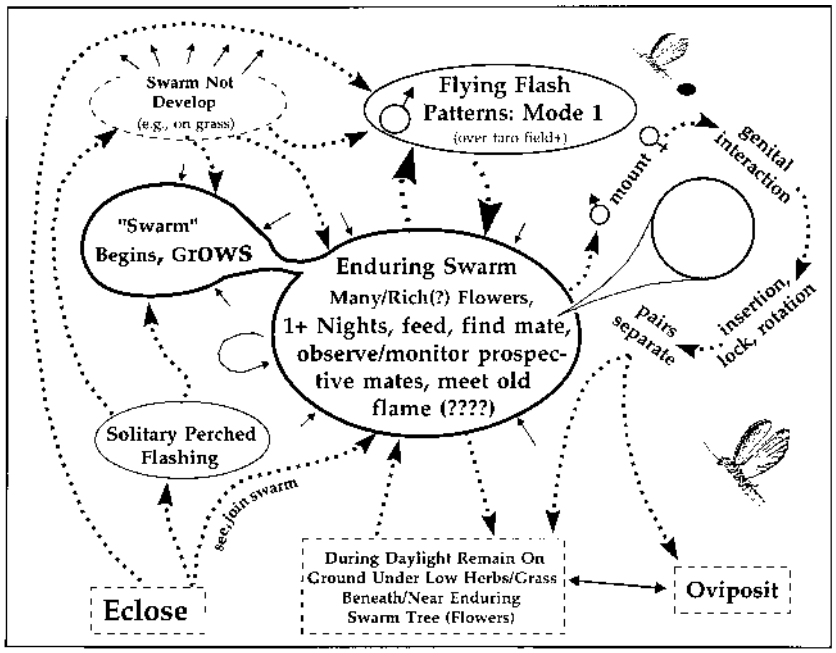


Fig. 9. Flow chart model of *P. pallens* sexual behavior, integrating observations at the ginger lily patch, the taro field, and other sites in the Comfort Castle-Millbank region along the Rio Grande River.

would connect with other insect behaviors I have found puzzling (Lloyd 1981). And, my thoughts return again and again to the fundamental taxonomist's question—just how many *P. pallens* species are there throughout Jamaica and her calendar?

ENDNOTES

I thank John Sivinski and Steve Wing for reading the manuscript. Florida Agricultural Experiment Station Journal Series Number R-06152.

The following enumerated statements are figure legends for color illustrations (slides) that appear as AuthorLink attachments to this article in the electronic publication of this issue of the *Florida Entomologist*, and which are cited in text here as ALR 1998, fig.#: 1. A view southeast along the gravel highway and upstream toward the Highlands. The Rio Grande River flows in the valley between the Blue Mountains and the John Crows. 2. Curved spikes on a jointers tree with feeding or sipping *P. pallens*. 3. A flashing *Photinus pallens* hanging and being wrapped in a spider web. The flashes of single fireflies in webs or on the ground, and even continuous emissions of light as from a flashlight attract *P. pallens*. 4. A patch of grass atop a hill above the Rio Grande River, where a few *P. pallens* gathered and flashed one evening. Apparently swarms that form at sites without many flowers do not become large nor long endure. 5. Flashing *Photinus pallens* at flowers on a spike in the grass. Though a few fireflies were attracted, large swarms were not seen at such sites. 6. A view of the ginger lily patch. Samples of flashing fireflies in this field indicate that 2000 or more may have

been present. Note the red plastic tags here and there. These mark flower spikes that were periodically sampled for firefly sexual activity. 7. A tagged ginger lily spike number 10, in the series of spikes that was sampled for sexual activity. 8. A mounted *P. pallens* male with extended aedeagus probing the abdomen tip of his mate to be. 9. The male in 8 and 9 (above) with partially inserted aedeagus. This connection seemingly indicates mate acceptance and requires the mechanical cooperation of both, though it is of course conceivable that males have some coercive leverage or that females can avoid using sperm that males have injected into them. 10. The connection (initiated in 8 and 9 above) is now complete, judging from external appearances, though inside the female's reproductive track there certainly are other significant events unfolding. 11. A pair partially rotated to a tail-to-tail position. 12. A pair has now completed rotated to a tail-to-tail position. Such pairs sometimes leave their flowers, where their lengthy(?) association presumably began, and remain on nearby leaves and bracts. Note the sexual difference in light organ topography.

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EXPLOITING THE INTERACTIONS OF CHEMICAL AND VISUAL CUES IN BEHAVIORAL CONTROL MEASURES FOR PEST TEPHRITID FRUIT FLIES

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ABSTRACT

Traps for tropical pest tephritids have relied primarily on chemical cues while traps for temperate pest tephritids have relied primarily on visual cues. Here we review research on the interactions between chemical and visual cues that have been observed in the development of traps for the tropical Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the temperate apple maggot, *Rhagoletis pomonella* (Walsh). By exploiting these interactions, it may be possible to produce efficacious trapping systems that could be used in a behavioral approach to fruit fly population control.

Key Words: Tephritidae, *Ceratitis capitata*, *Rhagoletis pomonella*, trapping, pheromone, bait

RESUMEN

Trampas para plagas de tefrítidos tropicales han dependido principalmente de señales químicas mientras que trampas para plagas de tefrítidos templados han dependido principalmente de señales visuales. Se revisan investigaciones sobre las interacciones entre señales químicas y visuales que se han observado en el desarrollo de trampas para la mosca del Mediterráneo, *Ceratitis capitata* (Wiedemann), de lugares tropicales y para la mosca de la manzana, *Rhagoletis pomonella* (Walsh), de lugares templados. Aprovechando estas interacciones desde un enfoque de comportamiento, es posible crear sistemas de trampeo eficaces para controlar poblaciones de moscas de la fruta.

Trapping systems for insects are important components in integrated pest management programs. Trapping data are used to make decisions on the initiation or termination of control measures, as well as to assess efficacy of control approaches that have been implemented. With the availability of sufficiently effective traps that capture both female and male pest insects, trapping systems may be used as behavioral control measures and, thus, could be added to the growing list of biologically-based technologies for insect control (U.S. Congress 1995). Adults of tephritid fruit flies use visual and olfactory stimuli to locate hosts (reviewed in Prokopy 1986), and both visual and chemical cues have been used in traps for pest tephritid fruit flies (reviewed in Cunningham 1989a, Economopoulos 1989). Traps for tropical tephritids, such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), have relied primarily on chemical lures (Gilbert et al. 1984), while the traps for temperate tephritids, such as the apple maggot, *Rhagoletis pomonella* (Walsh), have used visual cues (Prokopy 1968). In this paper, we will 1) review fruit fly traps that use either chemical or visual cues alone, 2) discuss the interactions that may occur among different cues associated

with traps for fruit flies, and 3) explore the ability to exploit the interactions between these cues to provide powerful trapping systems for pest fruit flies.

CHEMICAL CUES AND VISUAL CUES USED INDEPENDENTLY

Chemical Cues

Some of the earliest trapping systems for pest fruit flies relied on the use of baits made from proteins and fermenting sugar (Gurney 1925). Numerous substances have been tested, and a corn protein hydrolysate was found to be most effective for capture of *C. capitata* (reviewed in Roessler 1989) while yeast hydrolysates were found to be most effective for *Anastrepha* species (reviewed in Heath et al. 1993). These baits are usually deployed in McPhail traps (Newell 1936), which are bell-shaped invaginated glass traps with a water reservoir, or other bucket-type traps (reviewed in Cunningham 1989a). These protein-baited traps capture both female and male fruit flies. Male-specific synthetic chemical attractants have been discovered for tropical tephritids in the genera *Ceratitis* and *Bactrocera* (reviewed in Cunningham 1989b). These attractants are called parapheromones because they cause responses similar to true pheromones, but they are not produced by the responding species. Trimedlure, *tert*-butyl 4 (and 5)-chloro-2-methylcyclo-hexane-1-carboxylate (Beroza et al. 1961), is a highly effective, commercially available parapheromone for male *C. capitata*. Methyl eugenol (Howlett, 1915) and cue-lure (Alexander et al. 1962) are parapheromones that are attractive to males of oriental fruit fly, *Bactrocera dorsalis* (Hendel), and the melon fly, *Bactrocera cucurbitae* (Coquillett), respectively, as well as other *Bactrocera* species. Parapheromone lures are typically mounted in Jackson traps (Harris et al. 1971), which are white triangular cardboard traps that contain a sticky insert placed on the floor of the trap (Gilbert et al. 1984).

Visual Cues

Fruit flies use a number of visual cues to locate hosts, and appropriate visual cues may be highly attractive to pest Tephritidae (e.g. Prokopy 1968). Numerous studies have examined the effect of shape, size and color of visual stimuli on fruit fly response (reviewed in Katsoyannos 1989). Prokopy (1968, 1972, 1973) demonstrated that more *R. pomonella* were captured on fluorescent yellow rectangles and on enamel red spheres than on other shapes in different colors. He hypothesized that the flat surface of the rectangle together with the fluorescent color represented leaf-type stimulus that elicits food-seeking and/or plant-seeking behavior, whereas spheres constitute a fruit-type stimulus that elicits oviposition and/or mating-behavior. More *C. capitata* were captured on yellow rectangles than light orange, light green, red, gray or clear rectangles (Prokopy and Economopoulos 1976). Nakagawa et al. (1978) tested response of *C. capitata* to a wide variety of shapes and colors. In tests among 7.5-cm spheres of different colors, black and yellow captured the most females and black, yellow, red and orange captured the most males. Among spheres, cylinders, rectangles and cubes of equal surface area (175 cm^2) painted black or yellow, the black or yellow spheres caught the most of either sex. Among black or yellow spheres ranging in size from 1.5-to 18-cm diam, the black 1.5- and 3.2-cm spheres were two times more effective than equal sized yellow spheres, the black and yellow 7.5-cm spheres were equally effective, and the yellow 18-cm spheres were more effective than black 18-cm spheres. The yellow 18-cm spheres were most effective over all. Greany et al. (1977) found that fluorescent orange rectangles were the most effective for capture of the

Caribbean fruit fly, *Anastrepha suspensa* (Loew), and that most of the flies captured were sexually mature females (Greany et al. 1978). Sivinski (1990) found that more male *A. suspensa* were captured on 20-cm diam. orange spheres than spheres that were smaller or differently colored, but that female flies were trapped equally on 20-cm diam. green spheres. Green, yellow and orange were the most attractive colors for the Mexican fruit fly, *Anastrepha ludens* (Loew) (Robacker et al. 1990), but females preferred large spheres over large rectangles and small rectangles over small spheres (Robacker 1992).

Chemical Cues that Interact with Visual Cues

Addition of Visual Cues to Chemical Cue-Based Standard Traps

In the section above, we discussed examples in which the chemical cues and visual cues were used independently of other cues. Protein baits are often used in glass McPhail traps, so the only potential visual cue is the brown color of the bait. Similarly, trimedlure is widely deployed in white Jackson traps and can also be used successfully in clear traps such as a Steiner trap (Steiner 1957, Nakagawa et al. 1971). Studies have shown, however, that the addition of a visual cue to these chemical cues can increase fruit fly capture. Liquid protein-baited glass McPhail traps painted fluorescent yellow captured more fruit flies than unpainted McPhail traps or McPhail traps painted enamel yellow, red or gray (Prokopy and Economopolous 1975). There are several plastic McPhail-type traps used currently in fruit fly detection that use a yellow base as a visual cue (e.g., Katsoyannos 1994). Similarly, use of a fluorescent color insert instead of a white insert in trimedlure-baited Jackson traps increased *C. capitata* capture during certain times of the year in field trials conducted in Guatemala (Epsky et al. 1996). Trimedlure-baited yellow panels are used in a high-density trapping protocol when outbreaks of *C. capitata* are detected in the continental United States (Lance and Gates 1994), an example of combining a yellow visual cue with the chemical cue to optimize fruit fly capture.

Addition of Chemical Cues to Visual Cue-Based Standard Traps

There is a complex of visual cues and chemical cues emanating from a host tree that could provide improved capture of fruit flies, especially female fruit flies. Females travel to host trees to find both food and oviposition sites. Females require protein to ensure fecundity (Christenson and Foote 1960) and volatile chemicals released from protein baits provide food cues to foraging females. One of the chemicals released from protein bait is ammonia (Bateman and Morton 1981). Prokopy (1968) and Moore (1969) found that addition of ammonia to red spheres did not increase capture of *R. pomonella* over unbaited red spheres, however ammonia did increase capture on yellow rectangles. Prokopy (1972) hypothesized that the addition of ammonia to yellow rectangles increased fly capture because the yellow rectangle elicits food-seeking response and did not improve capture on red spheres because red sphere elicits primarily oviposition and mating-related behavior.

Host fruit odor is a potential source of chemical attractants for females looking for an oviposition site. *Rhagoletis pomonella* adults are attracted to the odor of fresh-picked apples in the field (Prokopy et al. 1973), and to synthetic apple volatiles in laboratory bioassays (Fein et al. 1982). In tests conducted in apple orchards, addition of synthetic apple volatiles increased capture of flies when the lure was added to red spheres, but not when added to yellow rectangles (Reissig et al. 1982). These exam-

ples demonstrate the importance of using the correct chemical cue and visual cue combination for optimal fruit fly trapping.

Use of Chemical and Visual Cue Interactions to Develop New Trapping Systems

Pheromone Volatiles—Many of the tropical tephritids have male-produced pheromones that could potentially be powerful, specific attractants for female flies. Although a number of putative pheromone components have been identified and have shown activity in laboratory bioassays, they have generally been less than satisfactory in field tests (Howse and Knapp 1996). An exception to this has been found with the papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker, which does not respond to food-type lures such as protein (Landolt 1984) or sugar (Sharp and Landolt 1984). Males produce a pheromone that is attractive to females (Landolt et al. 1985) and chemical analysis determined that it is composed of a single component (Chuman et al. 1987). Although female flies responded to synthetic pheromone in flight tunnel bioassays (Landolt and Heath 1988), attempts to capture papaya fruit flies with synthetic pheromone alone were unsuccessful. Field observations noted possible attraction to the chemical, but that flies would land on papaya fruit near the lure. However, by combining the pheromone lure with an appropriate visual cue, i.e. a green 12.7-cm diam. sphere that mimicked a papaya fruit, a trapping system for these flies was developed (Landolt et al. 1988). Subsequent research found that a pheromone-baited green cylindrical trap could be as effective as a pheromone-baited sphere (Heath et al. 1996a).

Over 60 components produced by calling male *C. capitata* have elicited electroantennogram responses in female *C. capitata* (Jang et al. 1989). Black spheres baited with three of the major components (ethyl-(*E*)-3-octenoate, geranyl acetate and *E*, *E*-α-farnesene) captured more females than unbaited spheres in field tests conducted in Guatemala (Heath et al. 1991). In subsequent flight tunnel bioassays, addition of a fourth component (△-1 pyrolline) increased response of female flies over the three component blend, however, response was less than response obtained with calling males (Heath and Epsky 1993). Field tests of black spheres and cylindrical traps baited with these synthetic blends captured few flies relative to traps baited with the protein bait (R. R. H. and N. D. E., unpublished). Thus, there may be additional chemical cues, visual cues or other cues needed to develop pheromone-based traps for female *C. capitata*. Presence of competing male fruit flies and host fruit may be complicating factors (Howse and Knapp 1996).

Food Volatiles—We have been involved in developing food-based synthetic attractants for tropical pest fruit flies. Initial research involved a two component synthetic attractant containing ammonium acetate and putrescine, and a cylindrical trap to protect the lures from the environment (Heath et al. 1995). In field tests conducted in Guatemala with wild populations of *C. capitata*, interactions between chemical cues and visual cues were an important aspect of trap and lure development. In tests of clear traps versus traps with a painted color strip (~7.5-cm high) around the periphery of the middle to provide a visual cue, more female *C. capitata* were captured in green traps than clear traps, with intermediate capture in orange or yellow traps. More male *C. capitata*, however, were captured in yellow traps than orange traps, with intermediate capture in clear or green traps. We then compared green and orange traps baited with a low, medium or high dose of synthetic attractant, and liquid protein-baited McPhail traps. In these tests, capture of females in both orange and green traps baited with synthetic attractant increased in relation to McPhail traps as dose of the synthetic attractant increased. Females captured in these studies were dissected to determine mating status. Throughout these tests, 21-25% of the females captured in the

McPhail traps were unmated. However, 55 and 69%, respectively, of the females captured in the orange and green traps baited with the low dose of synthetic attractant were unmated. In the same traps baited with the high dose of synthetic attractant, percent unmated dropped to 13 and 4%, respectively. Thus, both the sex and the reproductive state of the fly affected response to the visual and chemical cue combination.

Quantification of Chemical and Visual Cue Interactions

Studies conducted by Aluja and Prokopy (1993) quantified the interaction between visual cue (color of fruit model) and chemical cue (concentration of synthetic host fruit odor) in host finding by *R. pomonella*. They found that a direct relationship between fruit odor concentration and fruit fly ability to find baited clear spheres (weak visual cue), but that fruit odor concentration had no effect on fruit fly ability to find baited red spheres (strong visual cue). Thus a high degree of interaction among cues may indicate that the cues being evaluated could be improved further. For example, in our research to optimize cylindrical traps baited with a two component food-based synthetic attractant (ammonium acetate and putrescine), we found numerous interactions between visual cues and chemical cues (Epsky et al. 1995) and we used this information to optimize the trapping system (Heath et al. 1996b). Cylindrical traps with the painted surface on the interior of the trap (presenting a smooth, shiny exterior to the fly) were compared with traps with the painted surface on the exterior of the trap (presenting a rough, dull exterior to the fly). Orange and green traps were baited with the medium and high dose of the two component synthetic attractant, as were tested in previous research by Heath et al. (1995). Significantly more females were captured on dull green traps than on shiny orange traps at either dose and slightly more females were captured on dull traps versus shiny traps of the same color. Additional interactions were observed in tests with change in putrescine dose. In initial studies, putrescine was formulated using polypropylene vials (1-cm i.d., 2.2-cm long). The vial formulation was then compared to membrane-based putrescine lures with an exposed membrane opening of either 3- or 5-mm diam. The exposed membrane opening governs the chemical release rate, so the lure with the 5-mm opening releases a greater amount of putrescine than the lure with the 3-mm opening. The putrescine formulations were tested in green cylindrical traps with either a shiny exterior or a dull exterior that were baited with ammonium acetate lures. Traps baited with ammonium acetate and membrane-based putrescine lures captured the most *C. capitata* males and females. Visual cue and chemical cue interactions were observed in that the best capture among the traps with the shiny green exterior was with the 5-mm putrescine lure and ammonium acetate lure, but among the traps with the dull green exterior the best capture was with the 3-mm putrescine lure and ammonium acetate lure.

Subsequent research discovered that trimethylamine is a potent synergist to ammonium acetate and putrescine for capture of *C. capitata* (Heath et al. 1997). Traps baited with all three components captured more flies than traps baited with ammonium acetate and putrescine, and this was true whether it was tested in clear (glass McPhail traps), light green, dark green or yellow traps (Heath et al. 1997, Epsky et al. 1998). Thus, increase in potency of the chemical attractant by the addition of trimethylamine lessened the interaction with the visual cue used in the trapping system. Presence of a visual cue is still an important element in optimal trap performance for traps baited with the three component attractant, however, choice of visual cue is less critical.

Exploiting Interactions for Behavioral Control

Trapping systems have been developed primarily for use in detecting and monitoring target insects. There is an increasing need to move from insecticide-based control

measures to biologically-based control measures (U.S. Congress 1995), and the development of highly effective and selective trapping systems that target female fruit flies could provide a mechanism for behavioral control through mass trapping to be used alone and in conjunction with other integrated pest management systems. Experiments conducted in Greece indicated that populations of *C. capitata* could be effectively reduced when traps baited with liquid protein baits were deployed along with traps baited with trimedlure (Zervas 1996). Citrus fruit was protected from infestation by immigrating populations of *C. capitata* using mass trapping in combination with single-sex sterile male release (Economopoulos et al. 1996). In both studies, fruit was protected without insecticide application. Prokopy and Mason (1996) demonstrated protection of fruit from *R. pomonella* infestation by hanging sticky-coated red spheres in close proximity to synthetic fruit odor and synthetic food odor around the periphery of an apple orchard to intercept fruit flies immigrating into the orchard.

Although showing promise, these trapping systems do not provide the longevity necessary for use in long-term, mass trapping applications. Either sticky material or a water reservoir is used to kill attracted flies. Sticky surfaces quickly become deactivated by the accumulation of target and non-target insects on the surface, as well as by dust and debris that might be blown onto the trapping surface. Water-filled traps may dry out or become filled with captured insects. The ideal mass trapping system would last for 6-8 weeks and be essentially maintenance free during that time period. An alternative approach is the incorporation of a pesticide instead of the sticky material with the dry trap. A dry trap with the parapheromone methyl eugenol, mixed with a pesticide, was used successfully to eradicate the oriental fruit fly in a male-annihilation project (Steiner et al. 1965). Methyl eugenol is a feeding stimulant as well as an attractant. Thus, oriental fruit fly males consume the pesticide-laden formulation and obtain a lethal dose of pesticide. We developed a toxicant system that included a combination of visual cue, feeding stimulant and a pesticide in a formulation that could be applied in a relatively easy manner for use in traps as an alternative to sticky material (Heath et al. 1995). Panels coated with this material were placed inside a cylindrical trap to kill flies that have entered the trap. This toxicant system is deactivated if it is exposed to rain, thus compromising its use on the exterior surface of a trap (Duan and Prokopy 1995a, 1995b). Recent research has been directed towards the development of weather resistant, spatially localized toxicant-based bait stations. The incorporation of a pesticide with female-targeted synthetic attractants and well designed traps with appropriate visual cues into pesticide-bait stations would provide powerful tools not only for monitoring but potentially for fruit fly suppression that would avoid the environmental problems of pesticide bait sprays.

The availability of food-based synthetic attractants will afford a new dimension in exploring the interactions among visual cues and chemical cues for pest fruit fly females. Previous efforts with food-based liquid protein baits were hampered by batch to batch variability as well as by change in attractiveness of the bait over time (Epsky et al. 1993, Heath et al. 1994). Liquid baits require use of a trap with a reservoir, thus the ability to investigate interactions among chemical cues and visual cues using these lures is limited. Increased knowledge of behaviors associated with attraction of both sexually immature females and egg laying females will improve detection and delimitation of pest fruit flies, and provide increased protection of crops adversely affected by their presence.

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PHOTOTROPISM, BIOLUMINESCENCE, AND THE DIPTERA

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ABSTRACT

Many arthropods move toward or away from lights. Larvae of certain luminescent mycetophilid fungus gnats exploit this response to obtain prey. They produce mucus webs, sometimes festooned with poisonous droplets, to snare a variety of small arthropods. Their lights may also protect them from their own negatively phototropic predators and/or be used as aposematic signals. On the other hand, lights may aid hymenopterous parasitoids to locate fungus gnat hosts. The luminescence of mushrooms can attract small Diptera, and might have evolved to aid mechanical spore dispersal. Among Diptera, bioluminescence is found only in the Mycetophilidae, but the variety of light organs in fungus gnats suggests multiple evolutions of the trait. This concentration of bioluminescence may be due to the unusual, sedentary nature of prey capture (i.e., use of webs) that allows the "mimicry" of a stationary abiotic light cue, or the atypically potent defenses webs and associated chemicals might provide (i.e., an aposematic display of unpalatability).

Key Words: Mycetophyllidae, *Orfelia*, fungi, prey-attraction, aposematism

RESUMEN

Muchos artrópodos se mueven hacia o lejos de una fuente de luz. Larvas de ciertos moscos micetofilídos luminiscentes aprovechan este comportamiento para obtener sus presas. Estos moscos producen redes con una mucosidad, en ocasiones adornadas con gotitas venenosas, para atrapar a una variedad de artrópodos pequeños. Es posible que al mismo tiempo las luces los protejan de sus depredadores fototrópicos negativos y/o que las usen como señales aposemáticas. Por otra parte, las luces pueden ayudar a himenópteros parasitoides a localizar a los moscos micetofílidos. La luminiscencia de los hongos puede atraer a dípteros pequeños, pudiendo haber evolucionado para facilitar la dispersión mecánica de sus esporas. Entre los dípteros, la bioluminiscencia sólo se encuentra en los Mycetophilidae, pero la variedad de órganos luminiscentes que existe en esta familia de moscos sugiere una evolucion múltiple de esta característica. Esta concentración de bioluminiscencia quizá se deba a la forma, fuera de lo común, sedentaria de la captura de la presas (por ejemplo, el uso de redes) que permite el "mimetismo" de una señal luminiscente abiótica estacionaria, o a las atípicamente potentes defensas que sus redes y los productos químicos asociados pueden proveer (por ejemplo, una exhibición aposemática de ser desabridos).

Much of life, including flies, moves toward or away from light, an attribute that has interested both scientists and poets ("Ah sun-flower! Weary of time, / Who countest the steps of the sun," William Blake). In general, the mechanics of orientation to light have attracted more study than their functions; the functions often seeming self-evident. Mast (1911) provided an early list of plausible reasons for phototropisms, and included examples drawn from the Diptera: "Negative responses to light tends to keep these creatures (*fly larvae*) buried in the cadavers where they find food. . . . When . . . a bee in a flower or a pomace fly in a wormhole of a decaying apple is excited it flies directly to the light and ordinarily escapes."

In addition to simply moving towards shelter and darkness or freedom and light, arthropods also use light sources to navigate toward specific locations (the "light-compass reaction"). Bees use relative sun position to communicate food locations to their sisters (e.g., von Frisch 1967). Ants navigate with the aid of the sun in order to return along a "bee-line" to their nests (Santschi 1911). By keeping a constant angle to the sun (or to a pattern of polarized light in the sky generated by the sun) and taking into account the passage of time, certain ants can steer a straight course across even such complex and changing terrains as windswept desert sand. More interesting to the nocturnal student of bioluminescence, the moon is the light source used by at least two genera of navigating ants (Santschi 1923, Jander 1957). A beach dwelling amphipod, *Talitrus saltator* (Montague), also uses the moon, in this case to determine the direction towards optimum habitats (Papi 1960). On moonless nights the large yellow underwing moth, *Noctua pronuba* L., uses stars about 95 degrees from Polaris for navigation (Sotthibandhu & Baker 1979). When such an insect ". . . starts to fly across an area of 'unsuitable' habitat as part of a search for 'suitable' habitat it orients in it's individual-specific preferred compass direction." By avoiding 'wandering' it can cover the greatest possible territory with the least expense of time and energy (see also Baker 1978).

There can be dangerous consequences to positive phototropism and celestial navigation. A light, man-made or bioluminescent, can be mistaken for the greater illumination in a more open habitat. When a navigating insect confuses a small, nearby light source for a heavenly one, an attempt to keep the light at a constant angle rela-

tive to the body results in a spiraling flight into the source (e.g., Frankel & Gunn 1967). Flies can be both the victims and the beneficiaries of these mistakes.

Bioluminescent Adaptations in Flies

Luminous flies and prey attraction: Whatever the reasons for the orientation and movement of insects towards light, some bioluminescent Diptera have exploited the behavior for their own ends. All are fungus gnats (Mycetophilidae: Keroplatinae); Nematocera with vermiform larvae that resemble small crane flies as adults (Fig. 1a, d). Just a dozen or so of the more than 3000 species in the family are luminous, always as larvae, and often as pupae and adults; e.g., only the egg-stage of the New Zealand species *Arachnocampa luminosa* (Skuse) is nonluminous (Richards 1960), but adults and young larvae of the Swedish *Keroplatus sesiodes* Wahlerg bear no lights (Harvey 1952).

Most luminescent fungus gnats are poorly studied and some specimens remain officially undescribed. The latter include a single larva found on a New Guinea rain forest floor (Bassot & Hanson 1969, Lloyd 1978), an assembly of larvae once observed on the ceiling of a Nicaraguan cave (Gissele Mora, pers. comm.), a suspected new form of *Arachnocampa* collected in Fiji (Harvey 1952), and a spelunker's report of luminous "glow-worms" in an unidentified gypsum cave in the southwestern United States (Davis 1966: for a discussion of light organ placement and morphology see the section "Conclusion: the distribution of bioluminescence in flies").

The majority of mycetophilids develop in fleshy or woody fungi. Even those found in dead wood, under bark, or in the nests of squirrels and birds are probably mycetophagous (Vockeroth 1981). However, the larvae of luminous species are typically carnivorous. A possible exception to be addressed later is the Japanese *Keroplatus nipponicus* Okada (Kato 1953; in addition, see feeding habits of the German species *K. testaceus* Dalman [Pfeiffer & Stammer 1930, Stammer 1933]).

Luminescent species produce webs of mucous and silk. Web building is frequently encountered in both luminous and nonluminous carnivorous fungus gnats, (e.g., Mansbridge & Buston 1933). The strands of the web are often scattered with adhesive or poisonous droplets (i.e., the oxalic acid found in *Platyura* and *Orfelia* species). Generally, the larva has some sort of shelter associated with its web, either a connected crevice or a mucous tube. It ventures out to subdue small arthropod prey with a venomous oral secretion, and then retreats to the shelter with its meal. Larvae, acting in a manner reminiscent of spiders, restrain insects larger than themselves with mucous and later wrap them in silk. Rather than descend along the hanging strands of their webs, the larvae of *A. luminosa* swallow the line and pull their prey toward themselves (Richards 1960).

The forms of the webs vary substantially. That of *Orfelia fultoni* (Fisher) is a spray of strands suspended in a flat plane over hollow places on the surface (Fulton 1941; Fig. 1c). Spindle shaped deposits of adhesive anchor the side strands of the web, which may measure up to 5 cm across. The web of *A. luminosa* is suspended from the ceilings of caves and hollows, and consists of a horizontal thread from which are hung multiple "fishing lines" that can be more than 50 cm long in still, subterranean air, but are much shorter in more exposed situations (Gatenby & Cotton 1960; Fig. 1b). The lines are studded with adhesive droplets. A similar web is produced by a nonluminous species of *Orfelia, O. aeropiscator* Jackson, in the jungles and caves of Costa Rica (Jackson 1974), thus demonstrating that the very different plane-surface and suspended fishing-line designs can be generated by species within a single genus (see a discussion of the historical relationship of mycetophilid phylogeny and adaptation to saltation in evolutionary theory in Gould 1986, Goldschmidt 1948).

The prey of luminous fungus gnats consists of small arthropods, some of which presumably have been attracted by the predator's lights. *Arachnocampa luminosa* glows more brightly when hungry (Richards 1960). Larvae of this species in New Zealand's famous Waitomo Cave feed mainly on the chironomid midge, *Anatopynia debilis* (Hutton), that breeds in the waters beneath the glow-worm colony (Richards 1960). In other locations trogophytic tipulids, moths, stone flies, caddis flies, sand flies, red ants (apparently falling from the ceiling), spiders, millipedes, isopods, and even small snails are also captured (Stringer 1957). Cannibalism is common. Fulton (1941) found the remains of a cockroach and an ant in webs of *O. fultoni*, but noted that smaller insects were completely consumed and supposed that Collembola were its normal fare.

Transparent and blackened petri dishes covered with an adhesive have been placed over and near *O. fultoni* larvae in order to a) substantiate the hypothesis that larvae glow to attract prey and, b) to sample the insects attracted (Sivinski 1982). Collembola were commonly collected in both dark and illuminated traps, but only small Diptera, particularly cecidomyids and phorids, were significantly more numerous in traps baited with larval lights. The attraction of flying (i.e., mobile), but not of nonflying (i.e., relatively sedentary) arthropods, is consistent with the phototropic behaviors of the victims serving as a means of orientation during travel.

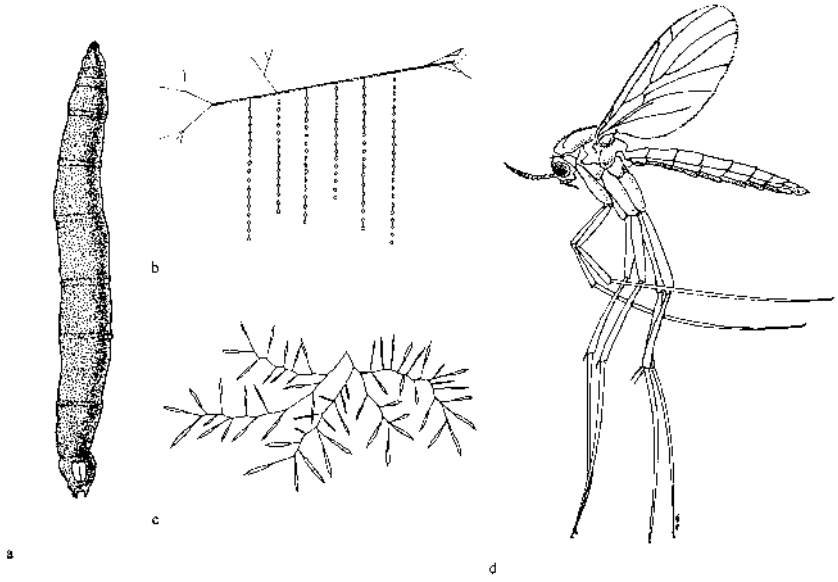

Fig. 1. a—The vermiform larva of *Arachnocampa luminosa* bears a single light organ on the terminal segment. Other species have lights on the head and tail (*Orphelia fultoni*), or glow along most of their bodies (e.g., *Keroplatus* spp.). b—Mycetophilids use various forms of webs to capture prey. In *Arachnocampa luminosa*, "fishing lines" are suspended from a major horizontal line connected to a larval retreat. c—The web of *Orphelia fultoni* is a flat spray of lines, typically spread over fissures in mossy soil. The lines are anchored to the substrate by adhesive droplets. d—An adult male of *Orphelia fultoni*.

The colors of mycetophilid luminescence differ from the usual greens and yellows of other insect lights (Sivinski 1981a). *Keroplatus sesiodes* and *japonicus* larvae emit a bluish-white light (Wahlberg 1849). *Orphelia fultoni* larvae, locally abundant in the damp ravines of the southern Appalachian Mountains where they are known as "dismal-lights," produce a vivid blue luminescence (Fulton 1939). *Arachnocampa luminosa* glows blue-green, with a peak emission at 487 nm (Shimura et al. 1966). Adult males, who seek out luminous female pupae and adults, have a corresponding peak in their visual sensitivity (Meyer-Rochow & Eguchi 1984; see section on luminous sexual signals). These unusual colors might contribute to prey attraction. Insect eyes tend to be more sensitive to the short wavelength colors, and Tyndall scattering may give a bluish cast to the celestially lite night sky, (unnoticed by human observers except "once in a blue moon"). If so, insect prey might perceive attractive, open, areas in foliage as being blueish.

Luminescence as a defense against predators: In addition to luring prey, bioluminescence may serve other roles. For instance, one Japanese fungus gnat, *K. nipponicus*, is both luminous and a web builder, yet it appears to eat only fungal spores (Kato 1953). Presumably, its light performs a function other than prey attraction, perhaps repelling negatively phototropic enemies (see Sivinski & Forrest 1983), or serving as an aposematic signal.

Mycetophilid larvae may not be defenseless. A web festooned with poisons and adhesives might alert a resident of a predator's approach or restrain it from reaching the larva. Fulton (1939) noted that webs woven by gregarious and nonluminous fungus gnats in decaying wood seemed to serve chiefly to block predatory or parasitic enemies (the luminous *K. sesiodes* is also gregarious, living in groups under a common glutinous web on the lower surface of mushrooms [Wahlberg 1848]; see also *K. tipuloides* Bosc [Santini 1982]). Cave wetas (Rhapdidophorids) in New Zealand caverns avoid the webs of *A. luminosa,* which tangle in their legs and antennae (Richards 1960). One unfortunate weta placed among webs remained corralled without food for sixteen days. A number of mycetophilid pupae are luminous (e.g., Gattenby & Cotton 1960, Sivinski 1982), and while these are unlikely to be engaged in prey attraction they might be emitting a warning signal. Attraction of food with light could be a secondarily evolved elaboration of what was initially an aposematic display and a fortress.

The luminescence of a number of fungus gnats, larvae and pupae, changes following disturbance (e.g., Gatenby 1959). This is consistent with lights repelling/startling negatively phototropic intruders, or acting as a warning signal. *Keroplatus testaceus* larvae brighten their lights when stimulated (Wahlberg 1849), although the glow of *K. nipponicus* remains constant despite "pressing, puncturing, and cutting" (Haneda 1957). *Orfelia fultoni* also continues to glow while its' container is handled (Fulton 1941). Tapping on the vial containing the unidentified New Guinean specimen increased the frequency of it's light emissions, but not its' intensity (Bassot & Hanson 1969). On the other hand, disturbances cause *A. luminosa* larvae to "gleam very brilliantly" for a brief time and then douse their lights (Hudson 1886, Gatenby & Ganguly 1956).

Miscellaneous luminous social signals: Manipulation of the phototropic responses of arthropods, including flies, is presumably responsible for the evolution of lights in mycetophilids. However, once evolved, lights could be used as displays in aggressive and sexual interactions.

i—Luminescence in larval conflicts: Light may communicate size and strength among conspecific larvae. Neighboring *A. luminosa* larvae commonly fight, the loser sometimes being eaten; "While fighting continues, each larva glows brilliantly, and it is comparatively easy to pick out a pair of fighting larvae in the darkness because of the intensity of the color and the brightness of their lights" (Richards 1960).

ii—Luminous sexual signals: Males of certain mycetophilid species orient towards adult-female and pupal lights to locate mates. While male pupae of *A. luminosa* are luminous (Gatenby & Cotton 1960), those of nearly mature females are particularly bright and likely to glow in response to touch (Richards 1960). An adult male landing upon a female pupa will cause it to luminesce. Males (up to 3 at a time) cling to such pupae, fighting to dislodge competitors and waiting for the female to eclose. If no male is attached at the time of eclosion, adult females employ light to ". . . attract a male fly, flashing it on and off till one arrived." Females usually lose their luminescence with the commencement of oviposition, though males continue to glow throughout their lives. The function of their continued luminescence is mysterious. Lloyd (1978) discusses the sexual selection of *A. luminosa*'s luminescent signaling system, and suggests that females, both as pupae and adults, may attempt to attract multiple suitors before copulating. The resulting competition among the males might result in inseminations by particularly fit mates. *O. fultoni* pupae are luminescent and adult males have been captured in traps baited with glowing larvae (Sivinski 1982). These larval lights may resemble luminous pupae to searching males.

The Dangers of Bioluminescence and Fungal Exploitation of Phototropism in Flies

Fly luminescence attracting predators and parasitoids: Luminous mycetophilids are attacked by hymenopterous parasitoids. An ichnuemonid, *Eusterinx* (= *Dalloterrea*) sp. emerged from a larva of *O. fultoni* (Fulton 1941), and a diapriid, *Betyla fulva* Cameron, was reared from the pupae of *A. luminosa* (Marshall 1882). Small unidentified Hymenoptera were most abundant in traps where *O. fultoni*'s larval lights were used as bait (Sivinski 1982). Two species of phalangids prey on the larvae of *A. luminosa* (Richards 1960). All 4 of the phalangids trapped in an *O. fultoni* habitat occurred in the ~1/4 of the traps where larval lights were visible, as did 10 of 18 spiders (Sivinski 1982).

Luminescent bacteria infect Diptera and other arthropods. For example, luminous, diseased chironomid midges have been observed across Europe, and in the New World, mosquito pupae in Brazilian epiphytes sometimes have glowing purple patches on their cuticles (cit. in Harvey 1952). Many infections appear to be benign, although some are fatal to their hosts. It is possible that bacterial lights attract new hosts, alternative hosts (e.g., fish) or agents of dispersal (Hastings 1978). For example, after entering an insect, one entomopathogenic nematode releases luminescent bacterial symbionts into the hemocoel. The microbes first kill the victim, and then luminesce (Nealson 1991). The light attracts other nematodes, which presumably carry the bacteria to another insect.

From a different perspective, nonluminous dipteran predators and parasitoids might locate luminous nondipteran prey by their lights. Adult North American fireflies (Lampyridae) of several genera are attacked by the parasitic phorid fly *Apocephalus antennatus* Malloch (Lloyd 1973), and to a lesser extent by a tachinid, *Hyalomyodes triangulifer* (Loew) (Sabrosky & Braun 1970, Lewis & Monchamp 1994). It is not known if the flies exploit bioluminescence to hunt down their hosts. However, host beetles occur in both luminescent and nonluminous genera (Brown 1994), and male and female *Photinus marginellus* LeConte, whose light displays differ considerably in frequency and duration, have similar rates of parasitism (Lewis and Monchamp 1994).

Luminous fungi and the exploitation of flies: A relationship based on positive phototropism may exist between luminous mushrooms and certain flies. Some fungi emit light. Luminescence can be present in mycelia (e. g., a number of *Mycena* species, Wassink 1978) or in both the mycelia and the fruiting body (e. g., North American populations of *Panellus stypticus* (Bull. Ex Fr.) Karst. (Cf. Singer), Buller 1924). Mushroom

(fruiting body) lights have been described as blue, white, or green depending on the species (Buller 1924, Wassink 1979). Emission intensities vary considerably. In the forests of Borneo *Mycena manipularis* (Berk.) Metrod are visible at 40 meters (Zahl 1971). One Australian species "pours forth it's emerald green light" with sufficient intensity to read by (Lauterer 1900 in Buller 1924). An American journalist wrote his wife from a World War II battle field in New Guinea; "I'm writing to you tonight by the light of five mushrooms." (Zahl 1965). Others, such as the common Floridian species *Dictyopannus pusillus* (Lev.), are dimmer and the eye often requires several minutes of dark adaptation before their glows can be perceived.

There have been a number of speculations on the function (if any) of fungal bioluminescence. For example, it has been suggested that the lights of mushrooms repel negatively phototropic fungivores, attract arthropods that then excrete in the vicinity of the fungus and so nurture it, and act as an aposematic display of distastefulness (at least one luminous species, the Japanese *Pleurotis japonicus* Kawam, is a common cause of human poisoning; cit. in Sivinski 1981b).

Perhaps the oldest of these hypotheses is that the lights attract spore-dispersers, i.e., insects that either contact and mechanically distribute spores, or feed upon and then defecate spores (Ewart 1906). The odor and colors of nonluminous stinkhorn fungi (Phallales) serve this role (e.g., Ramsbottom 1953). What rewards, similar to the stinkhorn's odoriferous, spore-laden "gleba," that luminous fungi might provide are unknown. If insects bear spores, it may be that they are simply manipulated by lights into contacting spore-bearing surfaces. Certain insects, particularly Collembola and small Diptera such as Phoridae, are more likely to be captured in glass traps baited with live, glowing *D. pusillus* than in traps containing freshly killed and dark specimens (Sivinski 1981b). Increased interactions with insects diminishes the plausibility of the argument that luminescence is a functionless, and by implication consequence less, byproduct of metabolism and is consistent with the attraction of spore dispersers.

The topography and timing of lights in fruiting bodies are suggestive of guiding dispersers. In *Mycena pruinosa-visida* Corner and *M. rorida* (Fr.) Quel. from the Far Eastern tropics only the spores glow (Haneda 1955). Most fruiting body lights are restricted to, or brightest, in the spore-bearing hymenium, i.e., "gills" (Wassink 1979), and *P. stypticus* glows most strongly at the time of spore maturation (Buller 1924). Interestingly, a number of fungal mycelia have a daily luminous rhythm, with minima around 9 o'clock in the morning and maxima around 9 o'clock at night (Berliner 1961a,b). Although spore dispersal is unlikely to be the function of these lights, their increased intensity during times when they can be best perceived suggests they play some communicative role in the biology of their emitters.

Conclusion: The Distribution of Luminescence in Flies

E. Newton Harvey, a giant in the study of bioluminescence, regarded the phyletic distribution of living light as its most puzzling aspect. He noted that while the number of luminous species is "vanishingly small," their diversity is surprisingly great; ". . ., as if a handful of damp sand has been cast over the names of various groups written on a blackboard, with luminous species appearing wherever a mass of sand struck." (1952). In the Diptera the "sand" only struck the Mycetophilidae.

The distribution of bioluminescence among flies presents a similar peculiar pattern. While luminescence is unique to single subfamily of fungus gnats, there is an extraordinary variety of light organ morphologies within the taxon. Lights are present in the anterior 5 segments and the small posterior segment of *O. fultoni* larvae, and consist of binucleate-giant-black, secretory cells (Bassot 1978). Species of *Keroplatus* tend to be luminous throughout their bodies, as was an unidentified New Guinean

larva whose glows traveled in waves along it's length (J. M. Bassot & F. E. Hanson 1969). In *Keroplatus* larvae the light originates from fat cells found around the gut (Kato 1953, Baccetti et al. 1987). The source of light in the New Guinean insect is unknown, although giant black cells were not present in the specimen (Bassot & Hanson 1969). The single light organ on the terminal segment of the abdomen of *A. luminosa* consists of modified Malpighian tube tissue and includes a concave mat of tracheoles on its' ventral side that acts as a reflector (Wheeler & Williams 1915). The various morphologies of fungus gnat light organs suggest several independent evolutions of bioluminescence within the family.

Why among flies did selection favor lights only in mycetophilids, but then so often? First, the step from nonluminescence to luminescence may not be particularly complicated, hence the potential for evolution to produce the great variety of luminous species (and fungus gnat light organs). In addition to the luminous Mycetophilidae, there are numerous instances of bioluminescent arthropods isolated from a phyletic history of bioluminescence; i.e., luminescence arising without sharing the genetic heritage of a recent luminous ancestor. For example, luminescent species of millipedes occur in only 2 genera, one Asian and the other restricted to certain mountain valleys in California (Haneda 1955, Causey & Tiemann 1969; see an odd case of luminous-milliped phobia in Yuswasdi [1950]). In the Coleoptera, hundreds of luminous species occur in families rich in bioluminescence such as the Lampyridae and Phengodidae. Yet only a single throcid (i.e., trixagid) species, *Balgus schnusei* Heller from French Guiana, is known to be luminescent. It emits green light from 2 swollen spots on the prothorax (Costa 1984; note that the Throcidae are related to the Elateridae, which contains a number of luminous species with similar prothorasic light organs [e.g., Lloyd 1978]). More surprising is the recent discovery of a luminous staphylinid! Costa et al. (1986) collected larvae of a Brazilian *Xantholinus* sp. with a light organ in the 8th abdominal segment. This is the only known case of luminescence in the entire Staphyliniformia, a clade of 28 families.

If bioluminescence can arise without extensive "preadaptation," why is it so rare or absent in many taxa? Or to turn the question around, what unusual set of circumstances favor its evolution in the fungus gnats, and not in other Diptera? Carnivorous mycetophilids are peculiar in that the larvae are nocturnal predators that employ webs to capture prey. Perhaps, such a stationary nature is both a requirement for duping phototropic victims and rarely encountered in flies (the pit-trap digging larvae of Vermilionidae are stationary, but underground and not visible to potential prey, e.g., Wheeler 1930). A slightly facetious critic of this argument might wonder why there are no bioluminescent spiders (although Brown 1925, 1926 reports a luminous spider in Myanmar [Burma] that glowed more brightly "when approached or shaken"). Alternatively, perhaps webs and their associated chemicals are one of the few potent defenses raised by relatively exposed dipteran larvae. If so, fungus gnat larvae may have rare opportunities to advertise their unpalatability to predators with light.

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BIOLOGY AND BEHAVIOR OF *PSEUDACTEON* DECAPITATING FLIES (DIPTERA: PHORIDAE) THAT PARASITIZE *SOLENOPSIS* FIRE ANTS (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Larvae of phorid flies in the genus *Pseudacteon* have the unusual habit of decapitating fire ant workers and pupating inside the empty head capsule which they use as a pupal case. Flies in this genus are the subject of considerable interest because they have the potential of being used as classical biological control agents against imported fire ants in North America. This paper details what is known and not known about their interesting life history, attack behavior, mating behavior, host specificity, and impacts on fire ant behavior. The biogeography, community structure, and possible impacts on fire ant populations are also considered.

Key Words: biological control, classical biocontrol, parasitoid, larvae, pupae, host specificity

RESUMEN

Moscas forídeas do gênero *Pseudacteon* produzem larvas que apresentam um habito não usual de decapitar as formigas operarias e pupas dentro da capsula cefalica vazia, a qual elas utilizam como câmara pupal. Estas moscas são asunto de consideravel interesse porque elas têm potencial de serem utilizadas como agentes de controle biológico classico contra as formigas lava-pé importadas na América do Norte. Este trabalho detalha o que é conhecido e desconhecido sobre seu ciclo de vida, com-

portamento de ataque, comportamento de reprodução, especificidade hospedeira e impactos do comportamento da formiga lava-pé. A biogeografia, a estrutura comunitaria e os possíveis impactos sobre as populações da formiga lava-pé são também relatadas.

Phorid flies in the genus *Pseudacteon* (Coquillett 1907) and several related genera (Pergande 1901) produce larvae that decapitate worker ants and pupate inside their empty heads. Not surprisingly, these miniature flies (Fig. 1) are about the size of the heads of their hosts. The attack behavior of *Pseudacteon* flies was first described in detail by Wasmann (1918) in Holland, Borgmeier (1922) in Brazil, and Smith (1928) in

Fig. 1. Lateral view of female *Pseudacteon nocens*. Length is about 1.4 mm.

the United States. Over a period of 50 years, Borgmeier gradually named most of the known species in this genus (Borgmeier 1925, 1962, 1963, Borgmeier & Prado 1975). The possibility of using these flies as fire ant biocontrol agents lead Williams (1980) to make extensive collections and observations in Brazil. Feener and Brown (1992) reported that *Pseudacteon* flies disrupted foraging of native fire ants in Central America and proposed that flies from South America might make good biocontrol agents for imported fire ants in the United States. Orr et al. (1995) and Porter et al. (1995c) documented substantial impacts of *Pseudacteon* flies on fire ant foraging in South America. The unusual life history of immature *Pseudacteon* flies was first described by Pesquero et al. (1995) and Porter et al. (1995b). Research groups at the University of Texas (Gilbert 1996) and the USDA-ARS laboratory in Gainesville, Florida are currently examining the potential use of *Pseudacteon* flies for classical biocontrol of imported fire ants in the United States.

LIFE HISTORY

The life cycle for *Pseudacteon* flies begins when a torpedo-shaped egg (Fig. 2A; Wasmann 1918) is injected into the body of a worker ant. The duration and morphology of the first instar is unknown, but the second instar is found in the ant's head by day four (Fig. 2B; Porter et al. 1995b). During this instar and most of the third instar (Fig. 2C), the maggot apparently relies on ant hemolymph for nutrition, because little

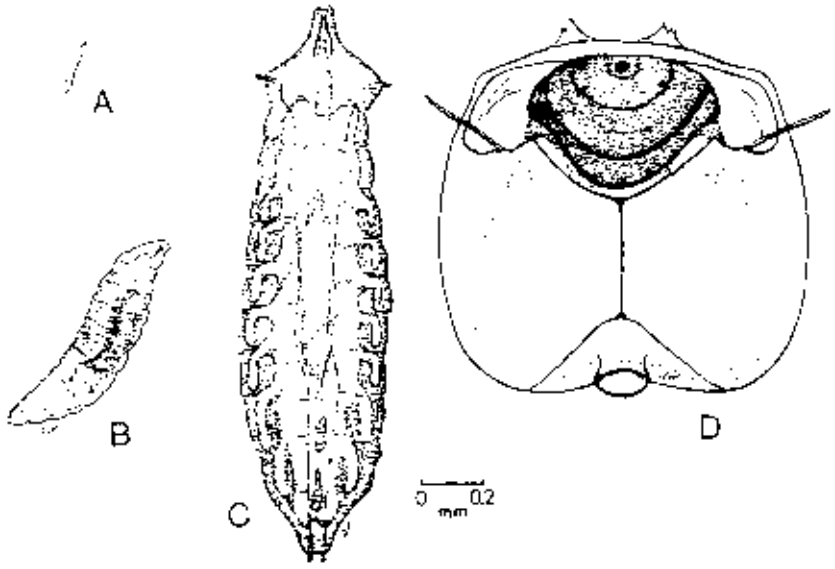

Fig. 2. Developmental stages of *Pseudacteon litoralis*. A) Egg, B) Second instar, C) Third instar. Note the two anterior spiracles projecting laterally behind the head region and the two paired posterior spiracles. D) Puparium inside head capsule of fire ant worker. The head capsule is shown in ventral view with the sclerotized cap of the fly puparium filling the mouth opening of its host. The remainder of the puparium is indicated by a dotted outline. Two respiratory horns extend diagonally out of the ant head capsule. (modified from Porter et al. 1995b)

if any tissue is consumed. Parasitized ant workers appear normal and healthy until a few hours before the maggot is ready to pupariate. It is not yet known what effects developing larvae may have on the behavior of their hosts; however, suppressing foraging would extend the longevity of their host (Mirenda & Vinson 1981, Porter & Jorgensen 1981) thus giving the larvae a better chance to complete development.

The decapitation process begins when parasitized workers crumple on their sides unable to walk (Fig. 3.1). The third instar maggot seems to release an enzyme or hormone that causes the intercuticular membranes of its host to degenerate (Porter et al. 1995b). This process usually loosens the head and first pair of legs; sometimes the other legs and gaster are also affected. The maggot then proceeds to consume the entire contents of the ant head, a 6-12 h process that usually results in decapitation of its living host (Fig. 3.2). The legs and sting of the headless body are often still twitching. In laboratory colonies, most decapitated and dying workers are rapidly carried out of the nest chambers onto the refuse pile. Using a series of hydraulic extensions (Fig. 4A), the maggot then pushes the ant's mandibles and tongue apparatus aside (Fig. 3.3). Eventually the maggot maneuvers itself under the tentorial arms inside the head capsule (Fig. 5). The first three segments then compress and harden to form a distinctive plate that precisely fills the oral cavity (Fig. 2D, Fig. 3.4). The remainder of the puparium remains unsclerotized and is protected by the ant head capsule (Fig. 5). Three to four days later, during actual pupation, two whisker-like respiratory horns emerge diagonally out of the puparium, positioned so that they extend out of the corners of the oral cavity of their host's head capsule (Fig. 2D and 5; Porter et al. 1997). This unusual type of puparium is shared by all 10 of the *Pseudacteon* species that have been reared to this stage (Porter et al. 1995b, Morrison et al. 1997, Porter et al. 1997; unpublished data).

The fate of *Pseudacteon* puparia in the field is not known, but based on laboratory observations, puparia are probably initially deposited with dead fire ant workers in refuse piles on the surface of the ground (Howard & Tschinkel 1976). Eventually the puparia are probably scattered by rain, wind and/or other species of scavenging ants. Pupal development requires 2-6 weeks, depending on temperature (Morrison et al.

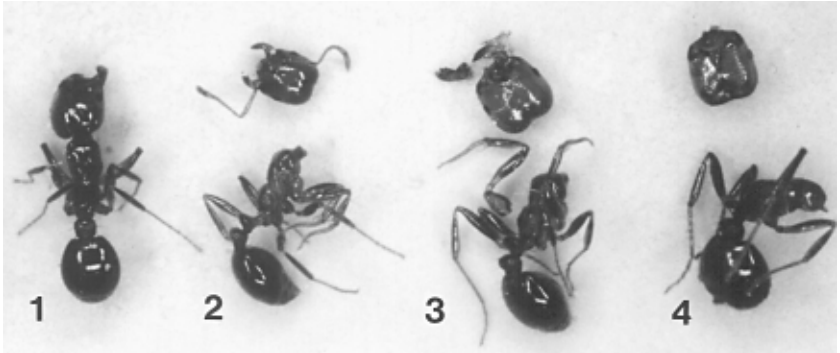

Fig. 3. Four stages in the decapitation of fire ant workers parasitized by *Pseudacteon* flies. 1) Crippled worker with degenerating intersegmental membranes and relaxed mandibles. 2) Decapitated worker with maggot consuming tissues in the head. 3) Ant head with mandibles and tongue apparatus pushed aside in preparation for pupariation. 4) Decapitated worker with fly puparium inside head.

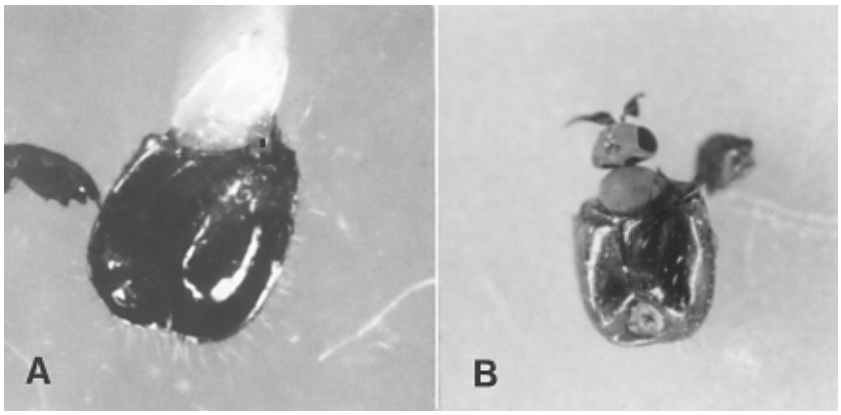

Fig. 4. A) Maggot pushing away ant mouth parts with a series of hydraulic extensions just prior to pupariation. B) Adult male fly in the process of emerging from puparium.

1997, Porter et al. 1997; unpublished data). The total developmental period from egg to adult is 5-12 weeks, again depending on temperature.

Emergence of adult flies generally requires only a few seconds (Fig. 4B). The sclerotized cap pops open and the adult fly slips out of the ant head capsule. Emergence only occurs in the first few hours after sunrise (Porter et al. 1997), as is the case for many kinds of flies. Newly emerged flies are ready to mate and lay new eggs within several hours of eclosion. Adult *Pseudacteon* flies are 0.9-1.5 mm in length (Borgmeier & Prado 1975), depending on the sex and species of fly. Adult flies can live 3-7 days in

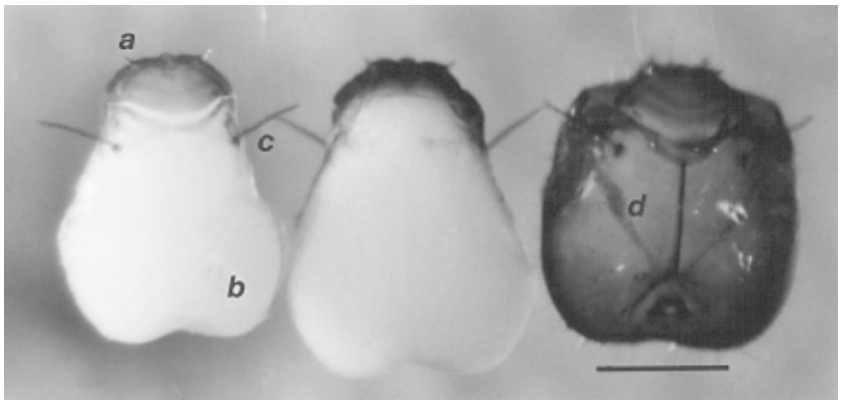

Fig. 5. Two *Pseudacteon* puparia removed from ant head capsules (dorsal and ventral views) together with puparium still in ant head capsule. Note the large white unsclerotized portion of the puparium that is normally protected by the head capsule of its host. a) Left anterior larval spiracle (compare Fig. 2c), b) Posterior larval spiracles (compare Fig. 2c), c) Pupal respiratory horns, d) Tentorial arms of ant extending diagonally across dorsum of fly puparium. Bar indicates 0.5 mm.

the laboratory if they are relatively inactive (Pesquero et al. 1995, Gilbert 1996, Porter et al. 1997). However, the life span of flies that are attacking ants is probably much shorter (Porter et al. 1997). Virtually nothing is known about what adult flies do or where they spend their time when they are not attacking fire ants. Adults will stop and drink water or lap up sugary substances if they contact them, but they do not appear to be attracted to them. *Pseudacteon* flies are not attracted to various kinds of fruits, flowers, human food products, or human feces (Porter et al. 1997; unpublished data). They are also not attracted to people. Data from Austin, TX indicates that adult flies commonly disperse several hundred meters from host colonies (Morrison et al. unpublished manuscript, University of Texas at Austin).

Interestingly, the sex of most *Pseudacteon* species seems to be facultatively determined by the size of the host (Fig. 6; Porter et al. 1997, Morrison et al. in press). This is probably because fire ant workers are highly variable in size (2-6 mm in length) and female flies are more fit if they emerge from larger hosts. The exact mechanisms of sex determination in *Pseudacteon* flies is unknown. Maternal sex determination via haplodiploidy occurs in many parasitic hymenoptera, but haplodiploidy is not known to occur in the family Phoridae or other related families of flies. Karyotypes should help resolve this question, as would transferring developing eggs or larvae from small hosts to large hosts and vice versa.

Attack Behavior

Female *Pseudacteon* flies hover 3-5 mm above their hosts while orienting for an attack (Fig. 7; Borgmeier 1922, Smith 1928, Williams 1980, Porter et al. 1995c). Once properly aligned, they dive in and inject an egg into the thorax of a worker ant using a hypodermic-shaped internal ovipositor (Wasmann 1918, Zacaro & Porter unpublished data). Each species of fly parasitizes a characteristic size range of ants (Morri-

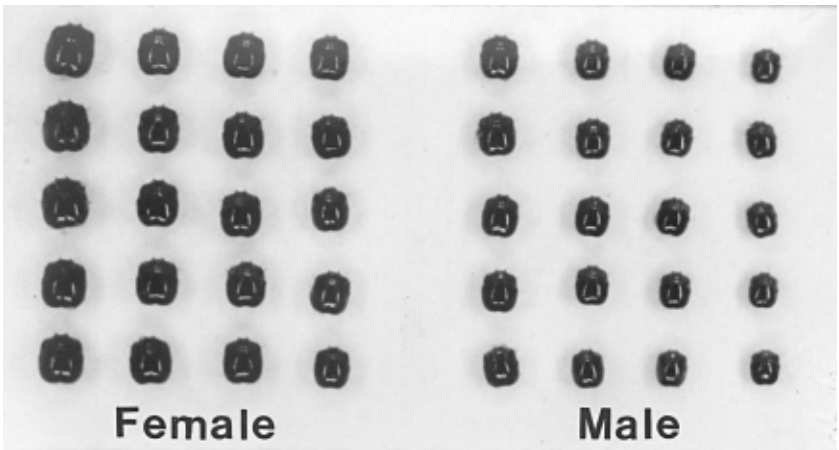

Fig. 6. Sex in most *Pseudacteon* flies appears to be determined by the size of their host. Female flies generally emerge from host head capsules that are distinctly larger than males. With *Pseudacteon tricuspis*, the lower quartile of female-producing head capsules overlaps with the upper quartile of male-producing head capsules. The width of head capsules ranges from about 1.3 mm (left) to 0.7 mm (right).

Fig. 7. Female *Pseudacteon* flies generally hover a few millimeters above their hosts prior to diving in and rapidly injecting an egg into the thorax.

son et al. 1997). This size range is usually consistent even across different ant species and colonies having different size ranges of workers (Morrison et al. in press, Morrison and Gilbert 1998). Male and female alates in the ant colony are ignored by most flies (Smith 1928, Williams & Banks 1987) and are never successfully parasitized (unpublished data). Egg laying bouts for *Pseudacteon tricuspis* Borgmeier and *Pseudacteon litoralis* Borgmeier generally last about an hour, during which time they attempt to oviposit 30-120 times (Morrison et al. 1997). Oviposition attempts result in parasitism 8-35% of the time depending on the species of fly and conditions (Porter et al. 1995b, Gilbert & Morrison 1997, Morrison et al. 1997, Porter et al. 1997). Female flies have 100-200 mature eggs in their ovaries upon emergence (Zacaro & Porter unpublished data).

Oviposition strikes are fast to very fast, requiring only 0.1-1.0 sec depending on the species (Borgmeier 1922, Porter et al. 1995a, unpublished observations). Each species of fly has a distinctively shaped external ovipositor (Fig. 8) which is presumably used in a lock-and-key fashion to align the internal ovipositor with a particular part of the host's body. The form of the external ovipositor varies greatly from species to species suggesting that each is used quite differently (Feener 1987). Unfortunately, the small size of the fly and the rapid speed of the attack has so far precluded any studies concerning the relationship between ovipositor form and function. The exact sites for egg injection are also not known, but the coxal region seems likely for most species.

Workers frequently appear stunned after an oviposition strike and often stilt up on their legs (Fig. 9A) for a few seconds to a minute before running away. The flies are generally too agile to be captured by fire ant workers; nevertheless, attacking fire ants is a dangerous activity. Only about 30% of female flies survive after 4 h of attacks in the laboratory (unpublished data). Many flies are apparently captured and killed when they accidently fall into clusters of ants. Other flies simply appear to run out of energy, stop flying, and are eventually chased down and killed by the ants.

How do flies locate fire ants? The answer is probably by cuing in on chemical odors (Borgmeier 1922, Donisthorpe 1927). When fire ant mounds are disturbed in South America, *Pseudacteon* flies usually begin appearing within 20 min if conditions are appropriate. Presumably, they are able to detect fire ant odors over long distances. Ex-

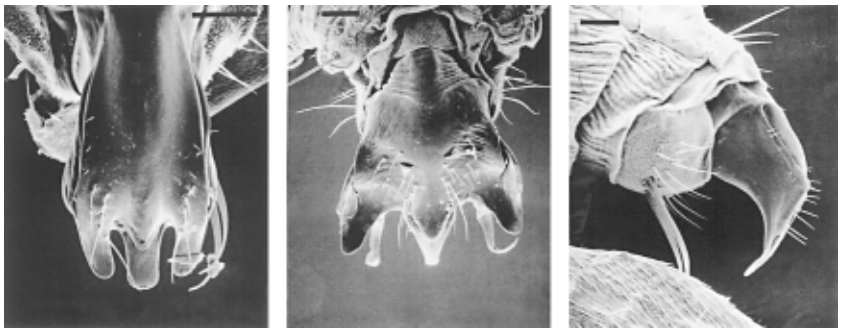

Fig. 8. Different species of *Pseudacteon* flies have very distinctive external ovipositors which are apparently used in a lock-and-key fashion to position the hypodermic-like internal ovipositor for injecting a single egg into their hosts. *Pseudacteon affinis* (left), *Pseudacteon tricuspis* from Argentina (center), *Pseudacteon borgmeieri* (right). Black bars indicate 50 μm.

actly how far is unknown; however, the fact that flies often require some time to appear suggests that they might be attracted from 10-20 m or more. However, studies of fly dispersal around Austin, TX suggest that flies are attracted at distances of less than 50 m (Morrison et al. unpublished manuscript). Chemical cues also seem important in the short-range recognition (10-40 cm) of fire ant workers. In the field in Brazil, several species of flies are capable of discriminating effectively and rapidly between *S. geminata* and *saevissima* complex fire ants (Trager 1991) at distances of 40 cm or more, even though workers are almost certainly visually indistinguishable at that distance (Porter et al. 1995a). It is not known whether long-range attraction cues are the same as the short-range recognition cues, but it seems likely. At distances

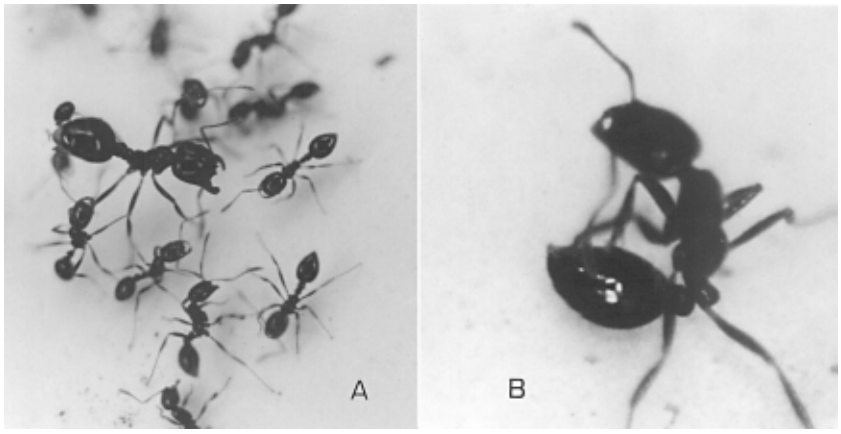

Fig. 9. A) After being attacked by decapitating flies, workers will often stilt up on their legs and remain immobile for several seconds to a minute as if they are stunned. B) When fire ant workers are being attacked, they often assume a stereotypical c-shaped defense posture.

of less than 10 cm, visual cues are probably very important. *Pseudacteon* flies have eyes with hundreds of ommatidia; presumably these afford them the necessary visual acuity to track, orient and attack fire ant workers. Nevertheless, even at 10 cm, odor cues appear to be necessary to initiate and maintain attack behavior (Porter & Alonso unpublished manuscript). The flies might also be able to use contact odors to assess the age and quality of fire ant workers. The source and nature of chemical cues are unknown, but alarm pheromones, recruitment pheromones, cuticular hydrocarbons, and aerosolized ant venom are all likely possibilities worth investigating (Orr et al. 1997).

MATING BEHAVIOR

In several *Pseudacteon* species (*P. tricuspis, P.crawfordi* Coquillett*, and P. browni* Disney) both sexes are attracted to fire ants and mating occurs while females are looking for workers to attack (Feener 1987, Feener & Brown 1992, Porter et al. 1997). Males can usually be distinguished from females because they are slightly smaller and because they do not track the movement of fire ant workers. Rather they hover in the air spinning around looking for females. Mating in *P. tricuspis* is initiated in the air when the male grabs hold of the female (Fig. 10). Copulation generally requires only a fraction of a second during which time the pair fall briefly to the ground before breaking up and flying away (Porter et al. 1997). Both sexes mate multiple times. The sex ratios of *P. tricuspis* adults collected in the field are often highly male-biased (e.g., 5:1, Pesquero et al. 1993; 2:1, Fowler et al. 1995, assuming all males were *P. tricuspis*). Males of most other species of *Pseudacteon* flies are not attracted to fire ants and their mating behaviors are currently unknown.

PSEUDACTEON BIOGEOGRAPHY

Pseudacteon flies have been collected in Europe, Asia, North America, and South America (Disney 1994, Michailovskaya 1995). At least 18 species of *Pseudacteon* flies have been found attacking *Solenopsis* fire ants in South America (Table 1). Another

Fig. 10. Male *Pseudacteon tricuspis* approaching female to mate while the female is searching for fire ant workers to attack. During mating, the pair generally fall to the ground where they remain in copula a few tenths of a second before breaking up and flying away. Black bar indicates 0.5 mm.

TABLE 1. *PSEUDACTEON* FLIES THAT ATTACK FIRE ANTS IN NORTH AND SOUTH AMERICA.

Species[1]	Known Range[1]	Ovipositor[1]	Abundance[2]
South America—*saevissima* complex ants			
P. borgmeieri	South America	unlobed	uncommon
P. convexicauda	Brazil	unlobed	rare
P. curvatus	South America	unlobed	common
P. solenopsidis	Brazil	unlobed	local
P. nudicornis	South America	bilobed	uncommon
P. affinis	Brazil	trilobed	rare
P. comatus	Brazil	trilobed	rare
P. cultellatus	South America	trilobed	rare
P. dentiger	Brazil	trilobed	rare
P. lenkoi	Brazil	trilobed	rare
P. litoralis	South America	trilobed	very common
P. nocens	South America	trilobed	uncommon
P. obtusus	South America	trilobed	common
P. pradei	Brazil	trilobed	common
P. near pradei	Brazil	trilobed	rare
P. species A	Brazil	trilobed	rare
P. tricuspis	South America	trilobed	very common
P. wasmanni	Brazil	trilobed	very common
Americas (Northern Hemisphere)—*geminata* complex ants			
P. crawfordi	U.S.A.	unlobed	
P. species B	U.S.A.	unlobed	
P. longicauda	Central America	unlobed	
P. antiguensis	Caribbean	bilobed	
P. browni	U.S.A., Central Amer.	bilobed	
P. grandis	Caribbean	bilobed	
P. spatulatus	U.S.A.	bilobed	
P. arcuatus	Caribbean, Costa Rica	trilobed	
P. bispinosus	Honduras	trilobed	

[1]Determined from Borgmeier & Prado 1975, Disney 1991, Disney 1994, specimens collected by and M. A. Pesquero and L. W. Morrison, and B. V. Brown's "scrapbook".
[2]Approximations from personal collecting efforts and (Williams 1980).

eight species attack fire ants in North America, Central America, and northern South America. Additional species will likely be discovered as collecting efforts are intensified and expanded into new areas. Also, several other species might need to be split if distinctive populations are determined to be separate species. (e.g., *Pseudacteon obtusus* Borgmeier and *P. tricuspis*). In contrast to the large number of *Pseudacteon* species that attack fire ants, only seven species (Disney 1994) are known to attack other

genera of ants in the New World (*Crematogaster, Linepithema, Dorymyrmex, Liometopum, Neivamyrmex*).

Most *Pseudacteon* species in South America are broadly distributed (Borgmeier & Prado 1975; unpublished data) across a wide range of habitats and climates. For example, *P. litoralis*, *P. tricuspis*, *P. obtusus*, and *Pseudacteon curvatus* Borgmeier have all been collected from São Paulo, Brazil in the north to Cuiaba, Brazil in the west, and south to Buenos Aires, Argentina. Even some of the less common *Pseudacteon* species (e.g., *P. borgmeieri, P. nudicornis* Borgmeier, *P. cultellatus* Borgmeier, *P. nocens* Borgmeier) have been collected from São Paulo south to Buenos Aires. These ranges encompass climates from tropical to temperate and habitats from tropical rain forests and swamps to temperate rangelands and seasonally dry "cerrado" forests. Several *Pseudacteon* species in North and Central America are also fairly widely distributed (Disney 1991).

Pseudacteon flies that attack fire ants appear to be associated with species in either the *saevissima* or the *geminata* complexes (Table 1; Borgmeier & Prado 1975, Gilbert & Morrison 1997, Porter 1998). Within both complexes, *Pseudacteon* species usually attack several species of fire ants (Disney 1994, Porter et al. 1997). However, phorid flies in South America have never been reported to attack the largest species of *Solenopsis* fire ants: *S. macdonaghi* Santschi, *S. megergates* Trager, *S. interrupta* Santschi, or *S. quinquecuspis* Forel. It will be interesting to determine whether these large fire ants lack *Pseudacteon* parasitoids, share them with their slightly smaller but more abundant relations (*Solenopsis invicta* Buren, *S. saevissima, Solenopsis richteri* Forel), or have their own, as yet undiscovered, communities of *Pseudacteon* flies.

Little is known about physical factors that limit the distribution of *Pseudacteon* species, but presumably there are thermal and moisture limits, as well as, limits associated with plant cover. Most decapitating flies do not seem restricted to specific habitats or narrow vegetational types. The abundance of *Pseudacteon* flies at particular sites can be quite variable from month to month, or even from week to week. The activity *Pseudacteon* flies around Austin, TX was limited by strong winds and stopped when air temperatures fell below 20°C (Morrison et al. unpublished data). Fowler et al. (1995) reported that *Pseudacteon* flies were active throughout the year in Rio Claro near São Paulo, Brazil with peak populations occurring in the spring. Populations in the fall can also be quite high (personal observations). There is no clear evidence for diapause or discrete generations in these flies, although species in Austin, TX do not appear to emerge during the winter months (Morrison et al. unpublished manuscript).

Community Structure

In South America, 5-8 species of *Pseudacteon* flies are often found at the same site (Porter et al. 1995a, Pesquero et al. 1996, Fowler 1997, Orr et al. 1997). At least three behaviors help explain how so many closely related species partition niche space while using the same host. First and perhaps most importantly, species in sympatric communities attack different sizes of fire ant workers (Fig. 11A; Campiolo et al. 1994, Fowler 1997, Morrison et al. 1997). When sympatric flies are viewed as a community, almost all sizes of fire ant workers are subject to attack from one *Pseudacteon* species or another (Morrison et al. 1997).

A second way that some phorid flies divide niche space is by selecting different periods of diurnal activity. In Brazil, *P. litoralis* is crepuscular, whereas the medium-sized *P. tricuspis* is most active from late morning until late afternoon (Pesquero et al. 1996).

A third way sympatric *Pseudacteon* species limit competition is that they attack fire ants engaged in different activities (Orr et al. 1997). For instance, some flies (i.e.,

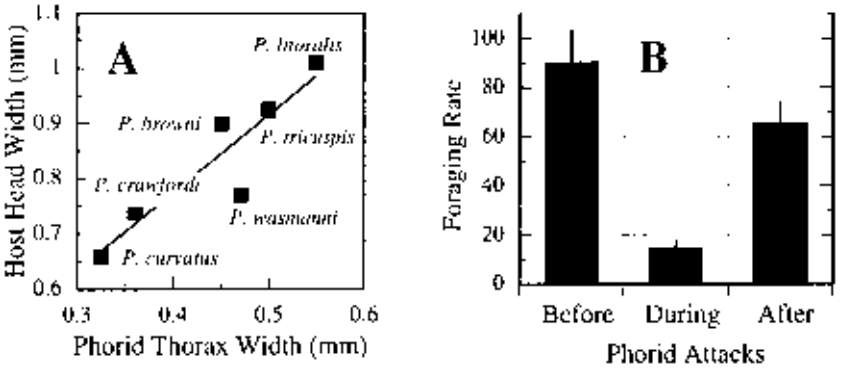

Fig. 11. A) Different species of *Pseudacteon* flies attack different sizes of fire ant workers. Often 5-8 species of flies occur at a single site. Taken together, species will attack >90% of fire ant workers (from Morrison et al. 1997). B) In South America, fire ant foraging generally terminates or is greatly reduced 2-3 minutes after decapitating flies begin attacking foraging workers. (modified from Porter et al. 1995c).

Pseudacteon solenopsidis (Schmitz), *P. borgmeieri, P. obtusus, P. nudicornis*) appear to specialize on fire ants along foraging trails (Orr et al. 1997) while other species appear to specialize on ants at mound disturbances or during fire ant mating flights (Smith 1928, Williams 1980, Pesquero et al. 1993). *Pseudacteon solenopsidis* has the interesting habit of chasing fire ant workers 10-20 cm off foraging trails before attacking them (Borgmeier 1922, Orr et al. 1995, Orr et al. 1997). This mode of attack is time-consuming; however, it may avoid shutting the foraging trail down (see below).

Impacts on Fire Ant Behavior

Fire ant workers are keenly aware of the presence of phorid flies (Borgmeier 1922). A single attacking fly usually stops or greatly inhibits the foraging efforts of hundreds of workers within 2-3 minutes (Fig. 11B; Feener & Brown 1992, Orr et al. 1995, Porter et al. 1995c). Orr et al. (1997) reported that the degree of response was related to the number of attacks. As soon as fire ant workers recognize the flies, they retreat into exit holes or find cover. Other workers will curl into a stereotypical c-shaped defensive posture (Fig. 9B; Feener & Brown 1992) that has only been reported when the ants are under attack by phorids. The c-shaped posture seems to be more common among *S. geminata* workers than *saevissima* complex workers (Feener & Brown 1992, Porter et al. 1995a, 1995c). Foraging rates usually remain suppressed as long as the flies are active and often for 15-60 minutes after the flies leave (Feener & Brown 1992, Porter et al. 1995c).

The flies inhibit fire ant foraging as long as they are present, often for periods of several hours (Orr et al. 1995). At any one time, phorid flies in South America can inhibit foraging at 10-20% of baits with fire ants (Porter et al. 1995c). Reduced foraging appears to facilitate competition from ant species that might otherwise be excluded from food sources in fire ant territories (Feener 1981, Orr et al. 1995). Several flies are also sufficient to stop nest construction or "freeze" the activity of entire colonies in laboratory nest trays (Fig. 12; Porter et al. 1995c). In Brazil, the "freezing" response varies from colony to colony (Porter et al. 1995c). Some colonies always show strong

responses while others show little or no response. Strangely, this variability was not related to collection location or species morphotypes.

The cessation of foraging, the c-shaped defense posture, and the freezing response are all specific fire ant behavioral defenses against phorid flies. Another probable defense is the foraging tunnel system (Disney 1994, Porter et al. 1995c). This system is a series of tunnels 2-7 cm below the ground surface that radiate out from the mound like branches on a tree (Markin et al. 1975). Even though a colony's territory may be 10 m across, foragers usually do not travel more than 0.5 m above ground from an exit hole. It would be difficult for fire ants to maintain large territories and therefore large colonies, if all foragers emerged from a central nest and traveled above ground for many meters with phorid flies attacking them. The tunnel system also allows colonies to shut down those portions of their territory under phorid attack while allowing them to maintain activity in the remainder.

The cues that fire ants use to recognize phorid flies are unknown. The ants can apparently see attacking flies and will clearly twist and turn to avoid their attacks. Olfactory and auditory cues might also be perceived by the ants at close range. Observations in the field indicate that hovering male flies can suppress foraging (Feener & Brown 1992, Porter et al. 1995c), but attacking females might be necessary to initiate defensive responses (Orr et al. 1997). If this is true, then fire ants may be releasing pheromones to trigger the group defensive responses.

HOST SPECIFICITY

All *Pseudacteon* flies are almost certainly parasitoids of ants. They have never been reported to attack any other kind of organism, and virtually all phylogenetically related phorid genera are also ant parasitoids (Brown 1993, Disney 1994). Their elaborate ovipositors and the adaptations of at least 11 species for pupation in the head capsules of worker ants (Fig. 2D) further supports the conclusion that they are very specialized parasitoids. Most *Pseudacteon* species are probably specific to ants in a specific genus (Disney 1994). A possible exception is *P. formicarum* in Europe. Donisthorpe (1927) reported that this fly attacks ants in several genera (*Lasius, Formica, Myrmica, Tapinoma*), but Wasmann (1918) held that it was probably specific to ants in the genus *Lasius*. Hosts of this fly have never been verified by rearing tests.

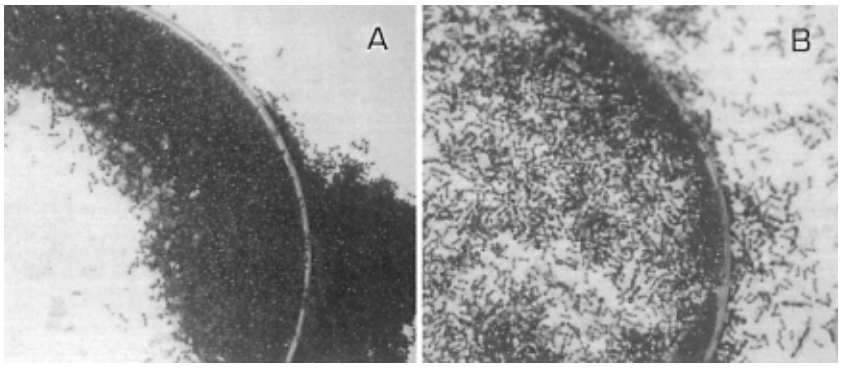

Fig. 12. A) If fire ants are unable to flee during attacks of decapitating flies, they will often "freeze" and refuse to move even when prodded. B) Normal colony activity with no flies present.

The *Pseudacteon* species that attack fire ants appear to be specific to fire ants. Of more than 20 New World species, only one unconfirmed report exists of a rare species being collected over another genus of ants (Borgmeier 1962). Some *Pseudacteon* species are apparently specific to individual fire ant species or species groups. For instance, at least three species of *Pseudacteon* phorid flies attack native *Solenopsis* fire ants in the U.S. (Table 1), but they have never been reported to attack imported fire ants even when they clearly have had the opportunity (Morrison et al. 1997). The host specificity of several parasitic *Pseudacteon* flies in South America was tested in the field with 23 species of ants from 13 genera (Porter et al. 1995a). As expected, these flies were attracted only to *Solenopsis* fire ants. A second field study showed that *Pseudacteon* flies are specific to ants in the genus *Solenopsis* (Porter 1998). Furthermore, several series of no-choice tests conducted in quarantine showed, that four species of *Pseudacteon* flies (*P. litoralis, P. tricuspis, P. wasmanni* (Schmitz), and *P. obtusus*) readily attack imported fire ants, but they virtually do not attack native fire ants (Gilbert & Morrison 1997, Porter & Alonso unpublished manuscript, Morrison & Gilbert unpublished manuscript). A fourth species (*P. curvatus*) does attack both native and imported fire ants, although it still has not been reared to the adult stage in native fire ants (Gilbert & Morrison 1997).

Impacts on Fire Ant Populations

The overall impact of phorid flies on fire ant populations is unknown; however, it is clearly sufficient to have caused the evolution of a number of phorid-specific defensive behaviors (Fig. 9B, 11B, and 12). These behaviors could only have evolved if *Pseudacteon* flies had exerted population-level impacts on the survival of fire ant colonies and/or their rates of sexual production (Porter et al. 1995c).

The introduction of exotic species usually occurs without natural enemies (DeBach 1974). This was certainly true for *S. invicta*. Over 30 natural enemies have been identified in South America (Williams 1980, Jouvenaz 1986, Porter et al. 1992) compared to only four in the United States (Collins & Markin 1971, Jouvenaz et al. 1977, Neece & Bartell 1981, Wojcik 1990, Kathirithamby & Johnston 1992, Williams et al. 1998).

The absence of natural enemies can allow exotic species to reach much higher population densities in newly invaded regions than in their native habitats (van den Bosch et al. 1973, Huffaker & Messenger 1976). Not surprisingly, fire ant populations in the United States are generally five times higher than in South America (Porter et al. 1992, Porter et al. 1997). Imported fire ants are one of the most abundant insects in the southeastern United States with average densities of 80-200 mounds/ha and 2,000-4,000 ants/m^2 (Macom & Porter 1996). Escape from natural enemies is a likely explanation for these unusually high densities, because analyses of factors such as climate, habitat, population structure, and cultural practices have not been useful in explaining intercontinental population differences (Porter et al. 1997).

Consequently, it is hoped that the introduction of phorid flies and other natural enemies from South America will be able to sufficiently tilt the ecological balance in the United States so that our native ants can compete with the imported fire ant on an "level playing field" (Fig. 13; Feener 1981, Feener & Brown 1992). If this happens, imported fire ant populations in the United States could be reduced to levels similar to those in South America. Phorid flies in North and Central America also have the possibility of being exported as biocontrol agents of exotic *S. geminata* populations in Africa, India and the Pacific region.

Ants are among the most important of all terrestrial arthropod groups in terms of both biomass and impacts on community structure (Hölldobler & Wilson 1990). Con-

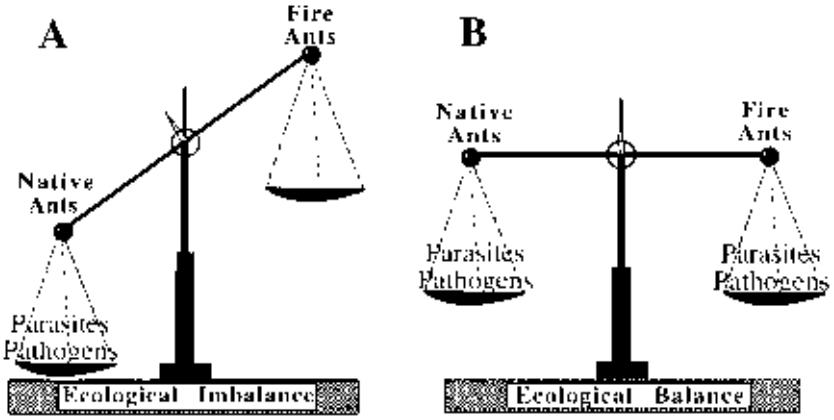

Fig. 13. A) One likely explanation for the unusually high densities of fire ants in the United States is that native ants are weighed down by their natural enemies while imported fire ants have escaped almost all of their natural enemies. B) Importing fire ant enemies that were left behind in South America may reestablish a more natural ecological balance. If this happens, fire ants will loose their competitive advantage and populations should drop.

sequently, there has been considerable interest in the structure and diversity of ant communities, most focused on competition among different species of ants (Wilson 1971). Relatively little, however, is known about the effects of pathogens and parasites on ant community structure (Feener 1981, Orr 1992, Briano et al. 1995, Orr et al. 1995), perhaps because experimental manipulations at the community level are usually very difficult or impossible to conduct. Fire ant biocontrol efforts offer a unique opportunity to experimentally test the hypothesis that parasitoids, specifically phorid flies, are important in structuring the diversity and composition of ant communities.

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THE MANIPULATION OF ARTHROPOD REPRODUCTION BY *WOLBACHIA* ENDOSYMBIONTS

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ABSTRACT

Wolbachia are intracellular bacteria that manipulate the reproduction of their arthropod hosts. The nature of the manipulation varies with *Wolbachia* strain, arthropod taxa, and arthropod genetic system. Nonreciprocal and reciprocal reproductive incompatibilities, sex ratio biases, and induction of thelytoky are some of the results of *Wolbachia* symbiosis. The *Wolbachia* present in the predatory mite *Metaseiulus occidentalis* are genetically similar to those found in insects, and are correlated with nonreciprocal incompatibility in crosses between infected males and uninfected females. The incompatibility phenotype includes reduced numbers of eggs, shriveled eggs, and a male-biased sex ratio of the few resulting progeny, which may be related to the parahaploid genetic system of this phytoseiid mite.

Key Words: symbiont, incompatibility, sex ratio, Phytoseiidae

RESUMEN

Wolbachia son bacterias intracelulares que manipulan la reproducción de sus hospederos artrópodos. La forma de esta manipulación varía con la sepa de *Wolbachia*, el taxón del artrópodo, y el sistema genético del artrópodo. Incompatibilidades reproductivas recíprocas y no-recíprocas, variaciones del coeficiente sexual, e inducción de telitoquia son algunos de los resultados de la simbiosis con *Wolbachia*. La sepa de *Wolbachia* que se encuentra en el ácaro depredador *Metaseiulus occidentalis* es genéticamente similar a la que se encuentra en insectos, y está correlacionada con incompatibilidad no-recíproca en cruzas entre machos infectados y hembras no infectadas. El fenotipo de incompatibilidad incluye la producción de un número reducido de huevos, huevos arrugados, y un coeficiente sexual de la poca progenie que resulta inclinado hacia el macho, el cual puede estar relacionado al sistema genético parahaploide de este ácaro fitoseido.

HISTORICAL OVERVIEW

Wolbachia are intracellular, transovarially-transmitted, rickettsial-like endosymbionts in the alpha-subdivision of the proteobacteria (purple bacteria). *Wolbachia* bacteria were first described from the gonadal tissues of the mosquito *Culex pipiens* L. in 1924 by Hertig and Wolbach. Unusual reproductive incompatibilities were described later in *Culex pipiens* mosquitoes by Ghelelovitch (1952) and Laven (1951). One type of these incompatibilities was nonreciprocal, meaning that crosses of males from population A with females of population B resulted in normal hybrid progeny, but crosses of males from population B with females from population A (the reciprocal cross) resulted in few viable hybrid progeny. Because the nuclear genetic makeup of the two hybrid crosses is essentially the same and the main difference is which mother's cytoplasm is interacting with the nuclear genes, the incompatibilities have a cytoplas-

mic inheritance pattern, also called "cytoplasmic incompatibility" (Laven 1959). In the 1970s Yen & Barr (1971) first correlated these nonreciprocal, cytoplasmic incompatibilities with the presence of *Wolbachia* endosymbionts. They found that when *Wolbachia*-infected males were treated with tetracycline (which is toxic to rickettsia-like microorganisms), they could reproduce successfully with uninfected females.

Because *Wolbachia's* morphological characters are of limited value and *Wolbachia* are difficult to culture outside the host (Weiss & Moulder 1984, O'Neill et al. 1992), their presence in other arthropods was merely speculative. However, in 1992, *Wolbachia*-specific polymerase chain reaction (PCR) primers were developed (O'Neill et al. 1992). These primers, designed to be specific to *Wolbachia*, are also general enough to amplify *Wolbachia* 16S ribosomal DNA from various insects. Reproductive anomalies associated with the presence of an unknown rickettsia could now be correlated with the presence of *Wolbachia*. For example, *Wolbachia* infection was confirmed in some California populations of *Drosophila simulans* Sturtevant (O'Neill et al. 1992). This symbiont was previously suspected as the causative agent of nonreciprocal reproductive incompatibilities between geographical populations of *D. simulans* (Hoffmann et al. 1986).

Wolbachia Biology

Wolbachia are transmitted through the egg cytoplasm, and therefore solely by females, except for one reported case of male transmission in laboratory populations of *D. simulans* (Hoffmann & Turelli 1988). *Wolbachia* are sensitive to high temperatures (Stevens 1989, Stouthamer et al. 1990, Girin & Bouletreau 1995, Louis et al. 1993), and the antibiotics rifampin and tetracycline (Stouthamer et al. 1990). The only success to date in culturing them outside the host has been in an *Aedes albopictus* (Skuse) cell line (O'Neill et al. 1995).

Because *Wolbachia* cannot be studied with traditional microbiological techniques (Weiss & Moulder 1984), techniques such as the polymerase chain reaction (PCR) and DNA sequencing have provided major breakthroughs in the study of these endosymbionts (O'Neill et al. 1992, Breeuwer et al. 1992, Rousset et al. 1992b, Stouthamer et al. 1993). The PCR allows the amplification of a specific region of *Wolbachia* DNA more than a million-fold. The presence or absence of the symbiont then can be determined by visual detection of the expected size fragment of DNA in an ethidium bromide-stained agarose gel under UV light. This amplification also yields ample DNA for sequencing and further description and characterization. DNA sequence analyses indicate a lack of concordance between the phylogenies of the symbiont and of the hosts, suggesting that this symbiont might sometimes be transmitted horizontally from species to species (Rousset et al. 1992b, O'Neill et al. 1992). Recent studies with the PCR determined that 16% of all insect species examined are infected with *Wolbachia* (Werren et al. 1995), and include representatives from a wide variety of orders and families (Giordano et al. 1997). For an extensive review of the current knowledge about *Wolbachia*, see Werren (1997).

Wolbachia's Effects

The effects of *Wolbachia* can be influenced by several factors. The strain of *Wolbachia* is important; some strains have been demonstrated to cause no reproductive alterations (Giordano et al. 1995). The phenotype of *Wolbachia*-mediated reproductive alterations also depends on the taxonomic status of the affected arthropod (Insecta, Arachnida, Isopoda) (see Werren 1997), as well as the genetic system of the arthropod.

It is important to consider arthropod genetic systems in order to better appreciate the diversity of *Wolbachia*'s effects on reproduction.

Diplo-diploid arthropods produce both sexes from fertilized eggs, each sex carrying both the maternal and paternal sets of chromosomes throughout their lives. In haplo-diploid arthropods, female progeny arise from fertilized eggs and are diploid, but the male progeny arise from unfertilized eggs and are haploid, carrying only the maternal set of chromosomes. Thelytoky is a genetic system in which virgin females produce diploid daughters parthenogenetically, rarely producing males. In a parahaploid genetic system, both sexes initially arise from fertilized (diploid) eggs, with both sets of chromosomes. However, one chromosome set is subsequently lost in males, and the adult male is haploid, producing sperm by a mitotic process.

When males of infected diplo-diploids mate with females lacking *Wolbachia*, the paternal chromosome set becomes abnormal in the fertilized egg (Kose & Karr 1995, O'Neill & Karr 1990), resulting in the death of both male and female progeny (Hoffmann et al. 1986, Hsiao & Hsiao 1985). The reciprocal cross is normal. Although the molecular mechanism of this incompatibility is not yet fully understood, it is speculated that *Wolbachia* somehow "imprints" or "modifies" the paternal set of chromosomes during spermatogenesis (Werren 1997), even though the bacteria themselves are not present in the mature male gametes. If *Wolbachia* is present in the egg cytoplasm, it can "rescue" the paternal chromosomes so that they remain normal and produce the normal diploid sons and daughters. If no *Wolbachia* is present, there is no "rescue" and those paternal chromosome set becomes abnormal, leading to embryonic death.

This same mechanism may occur in haplo-diploid insects, but with different consequences. When infected haploid males mate with uninfected diploid females, the male (haploid) progeny remain normal, but the normally diploid female embryos become haploid due to abnormalities in the paternal set of chromosomes (Ryan & Saul 1968, Reed & Werren 1995). The resulting phenotype of *Wolbachia*-mediated incompatibilities in haplo-diploid species is a strongly male-biased sex ratio because of the loss of female progeny. The haploid female embryos may die, as in some strains of the mite *Tetranychus urticae* Koch (Chelicerata: Arachnida) (Vala & Breeuwer 1996), or the haploid female embryos can become males thereby increasing the total number of expected males, as in the wasp *Nasonia vitripennis* Walker (Mandibulata: Insecta) (Breeuwer & Werren 1990, Ryan & Saul 1968).

Wolbachia can also cause bidirectional incompatibility in diplo-diploid species (O'Neill & Karr 1990) and haplo-diploid species (Perrot-Minot et al. 1996). In this situation, two populations apparently host two different *Wolbachia* strains. The result is reciprocal incompatibility, where both interpopulation crosses are incompatible.

Wolbachia induces thelytoky in some hymenopteran species, such as *Trichogramma* (Stouthamer et al. 1990) and *Aphytis* (Zchori-Fein et al. 1995). *Wolbachia* allows these females to produce diploid daughters parthenogenetically by causing gamete or chromosomal duplication early in the first mitotic division (Stouthamer & Kazmer 1994).

Wolbachia causes a typical diplo-diploid incompatibility phenotype in some isopods (Rousset et al. 1992a), as well as an unusual phenotype in the species *Armadillium vulgare* Latr. In this species, *Wolbachia* suppresses the androgenic gland in genetically male individuals, causing these male isopods to become functional females (Rigaud et al. 1991). It is likely that, with the diversity of *Wolbachia*'s effects on the arthropod taxa and genetic systems described to date, there may be more *Wolbachia*-mediated reproductive anomalies remaining to be described.

Because uninfected females are reproductively incompatible with infected males, and infected females can reproduce successfully with infected and uninfected males, infected females tend to have a reproductive advantage in polymorphic populations

(Caspari & Watson 1959, Turelli & Hoffmann 1991). The *D. simulans Wolbachia* infection has spread within and among California populations (Turelli & Hoffmann 1991, Turelli et al. 1992) since it was first documented in 1986 (Hoffmann et al. 1986). However, the ability of *Wolbachia* to spread through a population is modulated by various factors, including the stability of the infection as a function of maternal transmission frequency, fitness costs associated with infection, and the strength of incompatibility (Hoffmann et al. 1990, Turelli et al. 1992, Clancy & Hoffmann 1997).

The potential for *Wolbachia*-infected individuals to sweep through a population may be a useful phenomenon in the control of arthropod-borne pathogens. Efforts are under way to genetically engineer insects to be refractory to disease agents like those causing malaria or Chagas' disease (Beard et al. 1993). A mechanism is needed to enable these transformed arthropods to replace the wild-type insects already present in the field population. The ability of *Wolbachia* infection to spread through a population, as documented in *D. simulans*, could be harnessed as a mechanism to help drive a genetically altered trait through a population if the trait "hitchhikes" with the *Wolbachia*-infected cytoplasm (Caspari & Watson 1959, Beard et al. 1993). However, a fuller understanding of *Wolbachia* biology will be necessary before it can be used successfully as a drive mechanism (Werren 1997).

Wolbachia symbiosis may have other important effects. *Wolbachia*-mediated reproductive isolation may be one mechanism that could allow sympatric speciation to occur (Laven 1959, Werren 1997, Giordano et al. 1997). *Wolbachia* alters sex ratios and progeny survival and, as a consequence, may affect laboratory experiments and insect management in field programs. *Wolbachia* infection may have implications for mass rearing projects, especially if the bacteria have an influence on the quality of the natural enemies (Steiner 1993) or affect the rate of population increase of the individuals being reared.

Wolbachia in the Predatory Mite *Metaseiulus occidentalis*: A Case Study

The predatory mite *Metaseiulus* (= *Typhlodromus* or *Galendromus*) *occidentalis* (Nesbitt) is an important natural enemy of *Tetranychus* species, including *Tetranychus urticae* Koch. This predatory mite is used as a biological control agent in various crops in the western United States (Hoyt 1969, Flaherty & Huffaker 1970, Hoyt & Caltagirone 1971, Hoy 1985). Biological characteristics that affect these predators' rate of population increase are of particular importance (Sabelis 1985), including the presence of nonreciprocal reproductive incompatibilities between different strains or populations (Hoy 1985). Such nonreciprocal reproductive incompatibilities have been reported in *M. occidentalis*, and are associated with shriveled eggs, low numbers of eggs, low survival of immature stages, and reduced fecundity in surviving F_1 individuals (Croft 1970, Hoy & Knop 1981, Hoy & Standow 1982, Hoy & Cave 1988). Studies on the mode of inheritance of pesticide resistance have been affected by nonreciprocal cross incompatibilities (Hoy & Knop 1981, Hoy & Standow 1982). Nonreciprocal incompatibilities have interfered with hybridization studies between different phytoseiid mite populations (one method of determining species designations), including studies with *M. occidentalis* (Croft 1970), *Typhlodromus annectens* DeLeon (McMurtry & Badii 1989), and *Amblyseius addoensis* van der Merwe and Ryke (McMurtry 1980).

The cause of the nonreciprocal reproductive incompatibilities was unknown in these phytoseiids. An intracellular rickettsia-like microorganism was found by Hess & Hoy (1982) in *M. occidentalis* eggs and ovaries through light and electron microscopy. This observation, along with the nonreciprocal nature of the incompatibilities, led Hoy & Cave (1988) to speculate that a cytoplasmic factor may be responsible for

the observed reproductive aberrations seen in *M. occidentalis*, perhaps due to the presence of *Wolbachia*.

By using *Wolbachia* specific PCR primers which amplify the 16S ribosomal RNA and *ftsZ* genes, we determined that *Wolbachia* was present in both the predatory mite and its prey, *Tetranychus urticae* (Johanowicz & Hoy 1996). Unexpectedly, the *Wolbachia* DNA sequences from the two mite species were nearly identical to each other and to those from insects, including the type species *Wolbachia pipientis* from the mosquito *Culex pipiens*. Whether the *Wolbachia* from the mites are truly this similar to each other and to the symbionts from insects remains to be answered, because these genes are too conserved to resolve this question.

In order to study the biological effects of *Wolbachia* infection, it is crucial to obtain a population without the symbionts with which infected individuals can be crossed or compared. Rearing these mites at high temperatures (33°C) eliminated *Wolbachia* in the treated mites, as indicated by a PCR assay for infection status, and allowed crossing studies to be conducted. Interestingly, the incompatibility phenotype in *M. occidentalis* was a unique combination of reduced progeny production (as in diplo-diploids) and a skewed sex ratio (as in haplo-diploids) of the few resulting progeny, when infected males were crossed with cured females (Johanowicz & Hoy 1998). This phenotype may be due to the parahaploid genetic system (Hoy 1979) of this mite. Nelson-Rees et al. (1980) demonstrated cytologically that both male and female *M. occidentalis* are diploid at the beginning of embryonic development, but at the onset of the reductional division 24-48 hours after egg deposition, one of the sets (most likely the paternal set) becomes heterochromatinized and excluded from the nucleus, resulting in haploid males.

Conclusion

Wolbachia manipulate arthropod reproduction by causing nonreciprocal incompatibility, bidirectional incompatibility, skewed sex ratios, and thelytoky, depending on the *Wolbachia* strain, arthropod taxa, and genetic system. For example, *Wolbachia* is associated with both reduced egg production and a male-biased sex ratio of the few remaining progeny in *M. occidentalis,* a predatory mite with a parahaploid genetic system.

Interesting questions remain to be answered about *Wolbachia* symbiosis in *M. occidentalis* and in other arthropods. The exact mechanism of the reproductive manipulations remains unknown. A more detailed phylogenetic analysis of the *Wolbachia* in its various hosts using less conserved genes should provide a better estimate of the evolutionary relationships between the symbionts. Further study of *Wolbachia* infection dynamics may determine it's potential use as a drive mechanism. Other consequences of *Wolbachia* infection remain to be described. For example, Hsiao (1996) indicated that *Wolbachia* infection may be responsible for protecting the Western biotype of the alfalfa weevil from a parasitoid. Because of the complex interactions between this microorganisms and its arthropod hosts, a multidisciplinary approach will be helpful in answering many of these remaining questions.

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EFFECTS OF SUGAR/FLOUR SPHERES COATED WITH PAINT AND INSECTICIDE ON ALIGHTING FEMALE *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) FLIES

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ABSTRACT

We studied the behavior and fate of mature, wild-origin *Ceratitis capitata* (Wiedemann) females allowed to feed on 7-cm-diam spheres comprised of a mixture of sugar, flour and glycerin and coated with yellow latex paint containing either no insecticide, dimethoate (1.5% a.i.) or imidacloprid (1.5% a.i.). Females feeding on imidacloprid-treated spheres for 20 sec exhibited very little tendency to forage within host plants or to lay eggs either shortly after or 24 h after feeding, and suffered high mortality within 48 h. In contrast, females feeding on dimethoate-treated spheres for 180 sec exhibited, shortly thereafter, a tendency to forage within host plants and to lay eggs about equal to that of females feeding on untreated spheres, although they suffered high mortality within 24 h. In a field test, imidacloprid-treated sugar/flour spheres provided a significant level of protection of fruit from oviposition by *C. capitata* during 24 h periods (equal to that provided by sticky yellow spheres), whereas dimethoate-treated spheres did not. Further research on long-term activity of pesticide residue and on sphere performance under natural conditions will be necessary, however, before sugar/flour spheres coated with yellow latex paint and insecticide can be recommended for control of *C. capitata.*

Key Words: Mediterranean fruit flies, imidacloprid, dimethoate, spheres

RESUMEN

Estudiamos el comportamiento y el destino de moscas hembra maduras de *Ceratitis capitata* (Wiedemann) de origen silvestre a las que se les permitió alimentarse sobre esferas de 7 cm de diámetro compuestas de una mezcla de azúcar, harina y glicerina cubiertas con pintura de látex amarilla que contiene ya sea ningún insecticida, dimetoato (1.5% i.a.) o imidacloprid (1.5% i.a.). Las moscas hembra que se alimentaron en esferas con imidacloprid por 20 segundos exhibieron una muy baja tendencia a alimentarse en plantas hospederas o a poner huevecillos poco después de alimentarse o 24 horas después de alimentarse y sufrieron una tasa de mortandad alta dentro de un período de 48 horas. En cambio, las hembras que se alimentaron en esferas con dimetoato por 180 segundos exhibieron poco después niveles de tendencia a alimentarse en plantas hospederas y a poner huevos aproximadamente igual a los demostrados por las hembras que se alimentaron en esferas no tratadas con insecticidas, aunque sufrieron una tasa de mortandad alta dentro de un período de 25 horas. En un experimento de campo, esferas de azúcar y harina tratadas con imidacloprid proporcionaron un nivel significativo de protección a la fruta en contra de oviposición por *C. capitata* durante períodos de 24 horas (igual al que proporcionaron las esferas amarillas pegajosas), mientras que las esferas tratadas con dimetoato no lo lograron. Es necesario hacer investigación adicional sobre la actividad a largo plazo de residuos de pesticidas y sobre el funcionamiento de las esferas bajo condiciones naturales antes de que puedan ser recomendadas las esferas amarillas de azúcar y harina cubiertas con pintura de látex e insecticida para el control de *C. capitata*.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is an important pest of fruits and vegetables on several continents. A variety of traps has been developed for capturing *C. capitata* females and males (Heath et al. 1995), including sticky-coated fruit-mimicking sphere traps (Nakagawa et al. 1978; Cytrynowicz et al. 1982; Katsoyannos 1987; Katsoyannos & Hendrichs 1995). Yellow spheres have proven to be the most attractive colored spheres for *C. capitata* females, especially when 7 cm diam in size (Katsoyannos 1987).

Another tephritid, the apple maggot fly, *Rhagoletis pomonella* (Walsh), has been successfully controlled in commercial apple orchards using 8-cm-diam sticky-coated red wooden spheres hung (when unbaited) on every tree in an orchard or (when baited) on perimeter apple trees so as to surround an orchard (Prokopy & Mason 1996). Because considerable labor and expense are associated with cleaning such spheres every other week to maintain fly-capturing effectiveness (Duan & Prokopy 1995b), an alternative to sticky as the fly killing agent has been sought in the form of a mixture of pesticide, fly feeding stimulant and residue extending agent that could be applied to the sphere surface and kill alighting flies through ingestion of pesticide (Duan & Prokopy 1995b). A far less amount of pesticide is required to achieve mortality via ingestion than through tarsal contact alone (Duan & Prokopy 1995a). One shortcoming of this approach, however, has been rapid disappearance of fly feeding stimulant (sugar) during rainfall (Duan & Prokopy 1995a). To address this shortcoming, a new type of sphere has been created to replace wood as the sphere body (Hu et al. 1998). It consists of sugar entrapped in a mixture of gelatinized flour and glycerin. These ingredients are formed into a sphere, which is then dried and coated with a mixture of latex paint and insecticide. A sphere of this sort maintains a continuous supply of fly feeding stimulant on the sphere surface, even under rainfall, with latex paint acting as a residue extending agent for the insecticide (Hu et al. 1998). To date, two insecticides have shown more promise than any others tested when combined with latex paint applied to sugar/flour spheres: dimethoate (Duan & Prokopy 1995a) and imidacloprid (Hu & Prokopy 1998).

Here, we evaluated the potential of insecticide-treated yellow-colored sugar/flour spheres for use in controlling *C. capitata* females by comparing the effectiveness of dimethoate and imidacloprid. First, we asked which of these two insecticides ultimately yielded the greatest reduction in oviposition and the greatest mortality of alighting females. Next, we asked which of these two insecticides most strongly reduced intra-plant foraging and ovipositional activities of females between the time of alighting on spheres and the occurrence of mortality. Finally, we asked which of these two insecticides on spheres offered the greatest degree of protection of fruit against *C. capitata* oviposition.

Materials and Methods

C. capitata used in all greenhouse trials originated as larvae from infested fruit collected in Hilo, Hawaii. Upon eclosion, both sexes were maintained together in $30 \times 30 \times 30$ cm cages supplied with enzymatic yeast hydrolysate, sucrose and water until females were mature and tested at 14-21 days of age. Females were deprived of all food, but not water, 18 h before initial testing.

Spheres used in all experiments were similar to those described by Hu et al. (1998). Sucrose (60g) was dissolved in fructose syrup (55 ml), water (40 ml) and glycerin (20 ml), following which pregelatinized corn flour (50g) and wheat flour (50g) were added, mixed and heated in a microwave oven. The resulting dough was allowed to cool before it was formed into a 7-cm-diam sphere, threaded with a wire to facilitate

hanging. It was then dried in a regular oven, after which it received a coat of gloss yellow latex enamel paint (Glidden, Cleveland OH) as protectant. Then spheres received a second coating of the same paint containing either 1.5% a.i. of dimethoate (Digon 400, Wilbur-Ellis, Fresno CA), 1.5% a.i. of imidacloprid (Provado, Bayer, Kansas City, MO) or no insecticide, which we term dimethoate-treated, imidacloprid-treated or untreated spheres, respectively. Due to constraints of fly availability, we began testing one day after spheres received the second coating of paint. To elicit fly feeding response, 20% sucrose was added to the paint applied in the second coating. Three days are usually required for sufficient sucrose from the sphere body to penetrate paint and stimulate fly feeding (Hu et al. unpublished). For brevity, we hereafter consider the second coating simply as a mixture of latex paint and insecticide, not explicitly acknowledging the sucrose present in the mixture at application.

Greenhouse experiments were conduced in $70 \times 70 \times 70$ cm screen cages (open to the front), and protected above from direct sunlight with a covering of white paper. Each cage contained a small, non-fruiting potted coffee plant whose canopy was about 50 cm diam and had about 50 leaves. A sphere was hung near the front edge of the canopy. During 0900-1600 h, we released females singly onto the surface of a sphere, using a small piece of paper dipped in a 20% sucrose solution and attached to a probe to transfer the fly from a holding cage to the sphere. In the first greenhouse experiment, each female was allowed to remain on a sphere until it departed or fell due to poisoning. Total duration of stay and total time of feeding were recorded. Each fly was then transferred immediately to a 120 cm^3 plastic cup containing sucrose, water and an uninfested kumquat as an ovipositional site. After 48 h, the female was classified as being alive, dead or moribund (able to move but not crawl or fly and considered dead in data analysis) and the number of eggs laid was counted.

In the second greenhouse experiment, females were again transferred individually onto a sphere but allowed to feed only for a prescribed maximum amount of time, which was equivalent to the median duration of feeding in the first experiment: 220, 180 and 20 sec, respectively, for untreated, dimethoate-treated, and imidacloprid-treated spheres. Following feeding for this length of time or following departure or falling from a sphere (if a female left before reaching this allowable duration of feeding), we immediately transferred the female onto a leaf at the center of the plant canopy and removed the sphere from the cage. We recorded duration of fly stay on the plant (up to 15 min) and counted all leaves visited by flight or crawling within this period as a measure of foraging propensity. Thereafter, the female was transferred to a kumquat fruit hung from the plant. We counted all ovipositional bouts of the female during the next 5 min as a measure of propensity to oviposit. After this the female was transferred to a plastic cup with sucrose and water for 24 h, at which time females still alive were again assessed by repeating the above protocol.

In a field experiment, we compared the number of eggs laid by wild-population *C. capitata* females in kumquats protected by pesticide-treated or sticky-coated sugar/flour spheres or in unprotected kumquats. The experiment was conducted in a coffee plantation (on Kauai) harboring a moderate population of females that had virtually no access to natural oviposition sites because nearly all coffee berries had been picked or fallen. About 3 m from the end of each of 20 rows of coffee plants and about 10 m from the nearest neighboring test sites, we hung two uninfested kumquats about 6 cm apart, attached to branchlets by twist ties. We also hung two same-type spheres, each about 12 cm from the nearest kumquat. We cleared the area nearby of leaves to permit visibility of fruits and spheres. Each site was baited with an aqueous extract of ripe coffee fruit as an ovipositional attractant (Prokopy et al. 1997) and an aqueous solution of Nulure as a feeding attractant (Steiner 1952; Wakabayashi and Cunningham 1991). Solutions were applied to cotton dental wicks in separate glass vials. There

TABLE 1. BEHAVIOR, OVIPOSITIONAL PROPENSITY AND FATE OF *CERATITIS CAPITATA* FEMALES DURING OR AFTER EXPOSURE ON YELLOW PAINT/SUGAR-COATED SUGAR/FLOUR SPHERES IN GREENHOUSE ASSAYS.

Parameter Measured	No. Females Tested	Type of Sphere		
		Untreated	Treated with Dimethoate	Treated with Imidacloprid
Mean Duration of Stay (sec)[1]	20	564a	344b	238b
Mean Duration of Feeding (sec)[1]	20	333 a	231a	42b
Mean No. Eggs Laid when Confined with Kumquats during Next 48 h[1]	20	9.9 a	1.0b	1.0b
% Mortality after 48 h[2]	20	5	90	85

[1]Values within the same row followed by the same letter are not significantly different according to one-way ANOVA (following square root transformation) and the least significant difference test criterion at the 0.05 level. For mean duration of stay, $F = 7.67$, $df = 59$, $P \leq 0.001$. For mean duration of feeding, $F = 10.61$, $df = 59$, $P \leq 0.0001$. For mean number of eggs laid, $F = 23.99$, $df = 59$, $P \leq 0.000$.

[2]There is a significant difference among values in this row according to a Chi-square test for heterogeneity ($P \leq 0.0001$).

were five replicates of each of four treatments: no spheres, or sugar/flour spheres coated either with sticky, with paint containing 1.5% a.i. dimethoate, or with paint containing 1.5% a.i. imidacloprid. Initially, we included pesticide-free sugar/flour spheres as a fifth treatment. Unfortunately, on the first day, curious bypassers damaged some of these spheres. Because we had no replacements, we were obliged to begin the experiment anew without this treatment. Treatments within a replicate were rotated daily for 4 days i.e. until each treatment was at each site once. Kumquats were removed daily for counting eggs and replaced with fresh kumquats. Odor attractants were renewed daily.

All data obtained, except those analyzed as proportions, were subjected to square root transformation to stabilize variance. For data in Table 1, differences in percent mortality among treatments were compared using a χ^2 test for heterogeneity. All other data in Table 1 were subjected to one-way ANOVA. In Table 2, duration of fly residence on plants was divided into 3 groups (1-120 sec, 121-300 sec and 301-900 sec). Data were analyzed using χ^2 tests for heterogeneity. Other data in Table 2 were subjected to one-way ANOVA (for data 0 h after exposure) or Kruskal-Wallis nonparametric one-way ANOVA (for data 24 h after exposure). Field test data in Table 3 were subjected to one-way ANOVA.

RESULTS

In the first greenhouse experiment (Table 1), females stayed significantly longer on untreated than on dimethoate- or imidacloprid-treated spheres and fed significantly longer on untreated and dimethoate-treated spheres than on imidacloprid-treated spheres. During the next 48 h, under confinement with food and fruit, females that had been on untreated spheres laid about 10 times more eggs than females that had been on dimethoate- or imidacloprid-treated spheres. At 48 h, few females that had been on untreated spheres were classified as dead compared with females on insecticide/sugar treated spheres (Table 1).

TABLE 2. FORAGING BEHAVIOR OF *C. CAPITATA* FEMALES ON HOST PLANTS AND SUBSEQUENT OVIPOSITIONAL PROPENSITY AND FATE FOLLOWING FEEDING FOR 220, 180 OR 20 SEC, RESPECTIVELY, ON UNTREATED, DIMETHOATE-TREATED OR IMIDACLOPRID-TREATED YELLOW PAINT-COATED SUGAR/FLOUR SPHERES IN GREENHOUSE ASSAYS.

		Type of Sphere to Which Female Was Exposed before Transferred to Plant at							
		0 h after Exposure				24 h after Exposure			
Parameter Measured		No. Females Tested	Untreated	Treated with Dimethoate	Treated with Imidacloprid	No. Females Tested[3]	Untreated	Treated with Dimethoate	Treated with Imidacloprid
% of Females Staying on Plant for	120 sec[1]	15	100	100	53	14, 3, 8	93	67	100
	300 sec[1]	15	73	93	40	14, 3, 8	79	0	100
	900 sec[1]	15	40	80	20	14, 3, 8	71	0	100
Mean No. Leaves Visited[2]		15	7.0a	5.2a	0.6b	14, 3, 8	2.6	0.0	0.3
Mean No. Flights to Leaves[2]		15	6.1a	4.3a	0.6b	14, 3, 8	2.8	0.0	0.0
Mean No. Ovipositional Bouts[2]		15	0.9a	1.0a	0.1b	14, 3, 8	1.0	1.0	1.0
% Mortality after 24 h[1]		15	7	80	47	—	—	—	—

[1]For each row at 0 h, according to Chi-square tests for homogeneity, probability of a significant difference among values was $p \leq 0.0003$, 0.006, 0.004, and 0.0003, respectively, for 120 sec, 300 sec, 900 sec and % mortality; for each row at 24 h, $p \leq 0.189$, 0.002, and 0.005, respectively, for 120 sec, 300 sec, and 900 sec.

[2]At 0 h after exposure, values within the same row not followed by the same letter are significantly different according to one-way ANOVA (following square root transformation) and the least significant difference test criterion at the 0.05 level. For number of leaves visited, $F = 10.27$, $df = 44$, $P \leq 0.000$. For mean number of flights to leaves, $F = 2.15$, $df = 44$, $P \leq 0.002$. Foe mean number of ovipositional bouts, $F = 5.73$, $df = 44$, $P \leq 0.006$. At 24 h after exposure, probability of a significant difference (based on Kruskal-Wallis nonparametric one-way ANOVA) among values within a row was $p \leq 0.007$, 0.0001 and 0.126, respectively, for number leaves visited, number flights and number ovipositional bouts.

[3]Number females tested for untreated, dimethoate and imidacloprid spheres, respectively.

TABLE 3. PROTECTION OF KUMQUAT FRUIT BY PAINT/SUGAR-COATED SUGAR/FLOUR SPHERES AGAINST OVIPOSITION BY *C. CAPITATA* FEMALES IN THE FIELD.

	Mean No. Eggs Laid in Kumquats Protected by[1]			
No. Replicates Per Treatment	No Spheres	Dimethoate Spheres	Imidacloprid Spheres	Sticky Spheres
20	18.3a	14.5ab	7.4b	8.3b

[1]Values followed by the same letter are not significantly different according to the least significant difference test criterion at the 0.05 level. F = 3.50, df = 19, P ≤ 0.033.

In the second greenhouse experiment (Table 2), when assessed for propensity to forage on fruitless coffee plants immediately after feeding on a sphere for an amount of time equivalent to the median value observed in the first greenhouse experiment, females from imidacloprid-treated spheres behaved significantly different from females on untreated or dimethoate-treated spheres. The former visited only 11% as many leaves and made only 14% as many flights as females from dimethoate-treated spheres, which were not significantly different in these characteristics from females from untreated. Moreover, when exposed to kumquat fruit for 10 minutes upon departure or removal from a plant, females from imidacloprid-treated spheres engaged in only about 10% as many ovipositional bouts as females from dimethoate-treated or untreated spheres. At 24 h, only 7% of females from untreated spheres were dead compared with 80 and 47%, respectively, of females from dimethoate- and imidacloprid-treated spheres. When, in the second greenhouse experiment, females alive at 24 h post-exposure to spheres were re-evaluated for foraging propensity, essentially none of those from dimethoate- or imidacloprid-treated spheres visited any leaves by either flying or crawling (Table 2). Those from imidacloprid-treated spheres remained largely motionless. Numbers of ovipositional bouts per female were initially about the same as those found at 0 h after exposure to spheres for each treatment.

In the field experiment, imidacloprid-treated spheres protected kumquats over 24 h periods against oviposition by wild *C. capitata* females to a degree equal to that afforded by sticky spheres and numerically (although not significantly) better than that provided by dimethoate-treated spheres (Table 3). Among all tephritid females captured on the sticky spheres, 94% were *C. capitata*, suggesting a very high probability that the tephritid eggs in the kumquats were deposited by *C. capitata*, not by other tephritid flies.

DISCUSSION

Our findings indicate that sugar/flour spheres containing the insecticide imidacloprid at 1.5% active ingredient in the surface coating of yellow latex paint are highly effective in immediately immobilizing *C. capitata* females that alight and feed upon them for at least 20 sec. Such females were essentially unable to forage within host plants and had a low propensity to lay eggs either minutes after or a day after exposure to spheres. Nearly 50% died within 24 h and 85% died within 48 h of feeding. In contrast, females alighting and feeding for at least 180 sec upon sugar/flour spheres containing the insecticide dimethoate at 1.5% active ingredient in the surface coating of yellow latex paint were not immobilized immediately after feeding and in fact were able to forage within host plants and lay eggs equally as well as females that fed on sugar/flour spheres lacking insecticide. It was only after some undetermined amount

of time (but less than 24 h) following feeding on sugar/flour spheres containing dimethoate that females from such spheres suffered ill effects and a high probability of death.

Even though in the field experiment, imidacloprid-treated spheres offered a significant degree of protection of kumquats against egglaying by *C. capitata* over 24 h periods, whereas dimethoate-treated spheres did not, research needs to be carried out to determine if imidacloprid-treaded spheres have as much residual activity as dimethoate-treated spheres following the weathering action of rainfall and sunlight. In this vein, we did in fact exposed imidacloprid-treated, dimethoate-treated and untreated spheres to outdoor weather for 3 weeks following the experiments reported here but found that *C. capitata* females were very reluctant to feed on any of the spheres, even though to human taste, there was ample sugar on the sphere surface. A high proportion of the surface of each exposed sphere was covered with growth of microorganisms, which seemingly acted to deter fly feeding. These factors, along with identification of powerful odors to attract mature *C. capitata* females to yellow spheres (Katsoyannos et al. 1997; Prokopy et al. 1997), will need to be examined further to allow development of yellow sugar/flour spheres for potential direct control of *C. capitata.*

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APHIDS (HOMOPTERA: APHIDIDAE) COLONIZING PEACH IN THE UNITED STATES OR WITH POTENTIAL FOR INTRODUCTION

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ABSTRACT

Eleven aphid species known to colonize peaches in the United States and one species not known from the United States are described and illustrated. *Aphis spiraecola* Patch, *Brachycaudus helichrysi* (Kaltenbach), *Brachycaudus persicae* (Passerini), *Brachycaudus schwartzi* (Börner), *Hyalopterus pruni* (Geoffroy), *Hysteroneura setariae* (Thomas), *Macrosiphum euphorbiae* (Thomas), *Myzus cerasi* (F.), *Myzus persicae* (Sulzer), *Myzus varians* Davidson, *Pterochloroides persicae* (Cholodkovsky), and *Rhopalosiphum nymphaeae* (L.) are included in the present paper. A brief summary of taxonomic characters, usual hosts, and distribution within the United States are given for each species. Pictorial and dichotomous keys are included to aid personnel charged with detection, identification, and control of aphids associated with peaches in the United States.

Key Words: taxonomic keys, identification, control, distribution, *Prunus persica*

RESUMEN

Son descritas e ilustradas las once especies de áfidos que se conocen y que colonizan duraznos en los Estados Unidos y una especie que no se conoce en los Estados Unidos. Se incluyen en este trabajo a las especies *Aphis spiraecola* Patch, *Brachycaudus helichrysi* (Kaltenbach), *Brachycaudus persicae* (Passerini), *Brachycaudus schwartzi* (Börner), *Hyalopterus pruni* (Geoffroy), *Hysteroneura setariae* (Thomas), *Macrosiphum euphorbiae* (Thomas), *Myzus cerasi* (Fabricius), *Myzus persicae* (Sul-

zer), *Myzus varians* Davidson, *Pterochloroides persicae* (Cholodkovsky), y *Rhopalosiphum nymphaeae* (L.). Se presenta para cada especie un resumen breve de los caracteres taxonómicos, hospederos comunes, y su distribución dentro de los Estados Unidos. Se incluyen claves pictóricas y dicótomas para ayudar a personal encargado de la detección, identificación, y control de los áfidos asociados con los duraznos en los Estados Unidos.

Peaches (*Prunus persica* Siebold & Zuccarini) are widely grown for both commercial markets and home use. In 1992, more than 226,000 acres were under peach cultivation in the United States (Anonymous 1994). That same year, over 2.26 billion pounds of peaches were harvested with California responsible for more than half the total crop (Anonymous 1994). Preliminary data valued the 1995 peach crop at over $412 million (Anonymous 1996).

Several species of aphids can become established on peaches. When the colonies of aphids are large, they can greatly reduce plant vitality or even kill the plant through mechanical injury. This is especially true in a nursery situation. Feeding aphids also produce honeydew, a sticky substance that is potentially damaging. As aphids feed, honeydew is excreted and accumulates on the leaves and developing fruits. Honeydew can serve as a substrate for bacteria, yeast, and filamentous fungal growth which reduce plant vigor. Large amounts of honeydew on the developing fruit can cause splitting and fruit cracking (Barnett & Rice 1992) making the peaches unsuitable for the consumer market. Additionally, some aphids can transmit plum pox virus (= sharka disease) which affects peaches.

The aphid fauna associated with peaches in the United States includes at least 11 species that commonly colonize the trees. A brief summary of taxonomic characters, hosts, worldwide distribution, and U.S. distribution is given for each of the 11 species: *Aphis spiraecola* Patch, *Brachycaudus helichrysi* (Kaltenbach), *Brachycaudus persicae* (Passerini), *Brachycaudus schwartzi* (Börner), *Hyalopterus pruni* (Geoffroy), *Hysteroneura setariae* (Thomas), *Macrosiphum euphorbiae* (Thomas), *Myzus cerasi* (F.), *Myzus persicae* (Sulzer), *Myzus varians* Davidson, and *Rhopalosiphum nymphaeae* (L.). We are also including information on *Pterochloroides persicae* (Cholodkovsky), a known pest of peaches, other *Prunus* spp. (e.g. almond, apricot, and plum), and apples. Although currently not recorded in the United States, *P. persicae* has extended its range into Europe and northern Africa (Stoetzel 1990). Trade between these regions and the United States increases the chance for accidental introduction. Descriptions, figures, and keys are included as an aid for those responsible for detection, identification, and control of aphids associated with peaches in the United States.

Materials and Methods

In the synonymy section, one asterisk (*) represents the name used by Palmer (1952) and two asterisks (**) represent the name appearing in Blackman & Eastop (1984). Common names are those approved by the Entomological Society of America (ESA) (Stoetzel 1989).

Information on distribution and hosts is taken from labels on slides in the National Collection of Insects, Beltsville, Maryland, and from records in Palmer (1952), Smith & Parron (1978), and Blackman & Eastop (1984).

In the illustrated keys, the species are grouped by morphological differences in antennae and antennal tubercles, coloration of legs and abdomen, cornicles, shape of

cauda and number of caudal setae, and wing coloration and venation. Characters used in the keys are apparent with a dissecting microscope with a minimum power of at least 16X. Relative body size of aphid species follows the division proposed by Blackman & Eastop (1984): body length < 2.0 mm are "small," 2.0-3.0 mm are "medium," and > 3.0 mm = "large." Body length is measured dorsally from the center of the frons to the end of the abdomen, excluding the cauda (see generalized aphid, Fig. 1). Length of the antennal "terminal process" is measured as the distance between the large primary sensorium to the tip of the last antennal segment. Length of the "base" of the antenna is measured from the basal portion of the last antennal segment to the apex of the primary sensorium. Caudal length is measured along the midline from the beginning of the sclerotized portion to the tip (Fig. 1). Caudal width is measured between the hard and soft portion of the cauda (Fig. 1). The keys are not intended for identification of single, errant aphids but should be used for individuals fully colonizing peaches. Ideally winged aphids should develop from nymphs collected from a colony on the tree.

Aphis spiraecola Patch 1914
Figs. 1, 4, 5

Synonymy:
* *Aphis spiraecola* Patch
***Aphis citricola* van der Goot 1912
ESA approved common name: spirea aphid
Other common names: green citrus aphid

Taxonomic characters: Wingless adult female.—In life, body yellowish green to deep green, head brownish; tibiae pale to dusky with darker apical area. Small to medium sized, body length 1.8-2.1 mm, rounded. Frontal tubercles not well developed. Antennae 6 segmented, terminal process approximately 2⅓-2¾ times length of base of antennal segment VI; antennal segment III-V without secondary sensoria. Cornicles dark, without setae, tapered apically, 4-5¼ times as long as wide, slightly longer than length of cauda. Cauda dark, elongate, more than twice as long as wide with 3-5 pairs of lateral setae and 0-1 preapical seta.

Winged adult female.—In life, body yellowish green with head and thorax brownish; hind wing with 2 oblique veins. Small to medium sized, body length 1.7-2.3 mm, rounded. Antennae 6 segmented, terminal process approximately 2¼-3 times length of base of antennal segment VI; antennal segment III with 6-8 secondary sensoria, antennal segment IV with 0-2 secondary sensoria; antennal segment V without secondary sensoria. Cornicles dark, elongate, without setae, tapered apically, 2¾-4 times as long as wide, slightly longer than length of cauda. Cauda dark, elongate, more than twice as long as wide with 3-7 pairs of lateral setae and 0-1 preapical seta.

Hosts: Polyphagous, over 20 families recorded as hosts, especially Asteraceae, Caprifoliaceae, Rosaceae including *Prunus* spp., Rubiaceae, and Rutaceae.

U.S. distribution: Widespread.

World distribution: Virtually worldwide.

Comments: *Aphis spiraecola* transmits 17 plant viruses (Chan et al. 1991) including plum pox virus, which affects peaches.

Brachycaudus helichrysi (Kaltenbach 1843)
Figs. 1, 6, 7

Synonymy:
* *Aphis helichrysi* Kaltenbach
** *Brachycaudus helichrysi* (Kaltenbach)

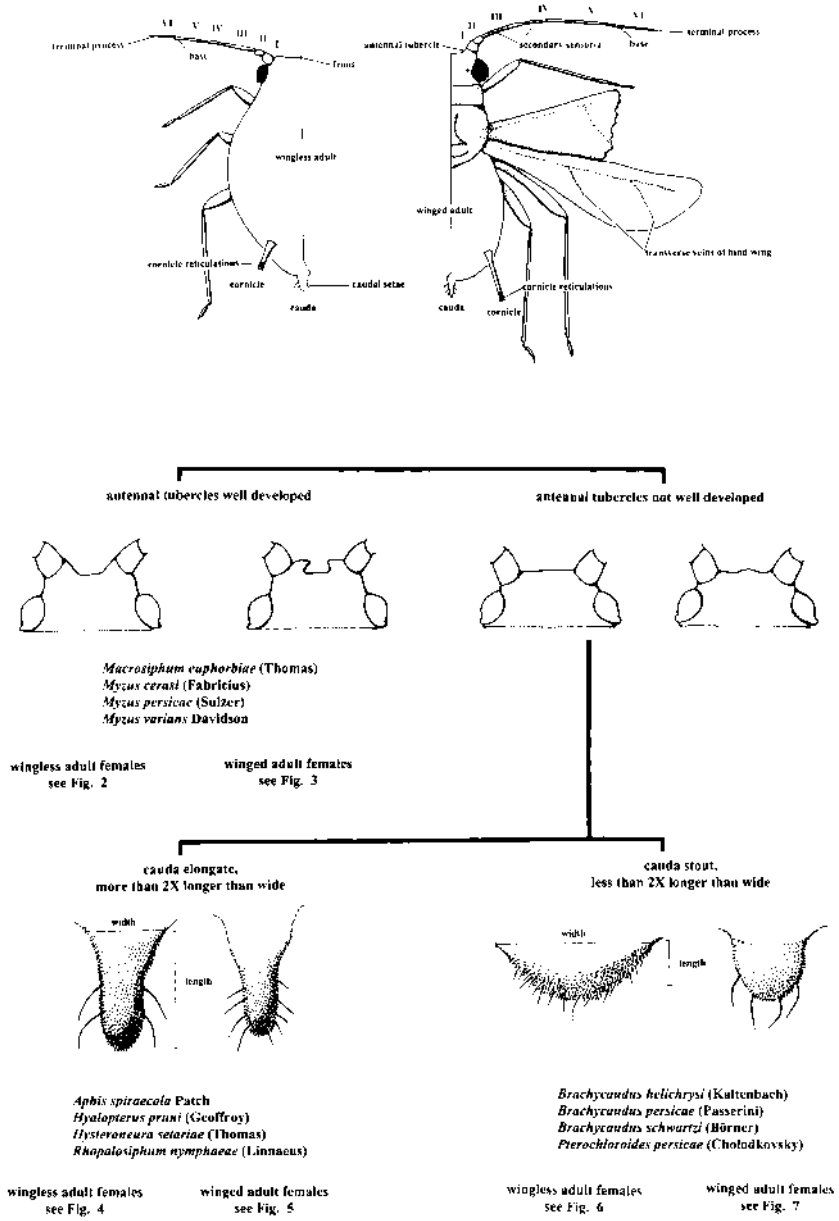

Fig. 1. Pictorial key to 12 aphid species that potentially colonize on peaches in the United States.

ESA approved common name: none

Other common names: peach leaf curl aphid, leaf-curl plum aphid, leaf-curling plum aphid.

Taxonomic characters: Wingless adult female.—In life, body color varying from green to yellow to nearly white or sometimes pink; legs pale. Small to medium sized, body length 1.7-2.1 mm, pear shaped. Antennae 6 segmented; tubercles not well developed; terminal process approximately 2-2½ times length of base of antennal segment VI; antennal segment III-V without secondary sensoria. Cornicles pale, apically dusky, without setae, slightly tapering to a constricted area near apical flange; approximately 1¼-1⅔ times as long as wide, subequal or longer than length of cauda. Cauda pale, stout, less than twice as long as wide with 2-3 pairs of lateral setae and 1 preapical seta.

Winged adult female.—In life, body shape and coloration similar to wingless adult female but abdomen with a dark dorsal patch that is usually confined to posterior several segments; antennal segments I-VI dusky on slide-mounted specimens; small sized, body length 1.2-1.4 mm. Antennae 6 segmented; tubercles not developed; terminal process approximately 2⅔-3⅔ times length of base of antennal segment VI; antennal segment III with 17-23 secondary sensoria; antennal segment IV with 1-7 secondary sensoria; antennal segment V without secondary sensoria. Cornicles completely dusky, without setae, slightly tapering to a constricted area near apical flange; approximately 2-3¼ times as long as wide, subequal or longer than length of cauda. Cauda pale, stout, less than twice as long as wide with 2-3 pairs lateral setae and 1 preapical seta.

Hosts: Primary hosts are *Prunus* spp.; secondary hosts include species of various Asteraceae, Boraginaceae, and sometimes *Salix*.

U.S. distribution: Widespread.

World distribution: Virtually worldwide.

Comments: *Brachycaudus helichrysi* transmits nine plant viruses (Chan et al. 1991) including plum pox virus.

Brachycaudus persicae (Passerini 1860)
Figs. 1, 6, 7

Synonymy:

* *Aphis persicae-niger* E. F. Smith 1890a, b

** *Brachycaudus persicae* (Passerini 1860)

ESA approved common name: none

Other common names: black peach aphid

Taxonomic characters: Wingless adult female.—In life, body color shiny black with anterior and lateral areas brown; legs yellow to dusky. Small to medium sized, body length 1.7-2.1 mm, pear shaped. Antennae 6 segmented; tubercles not well developed; terminal process approximately 4-5 times length of base of antennal segment VI; antennal segment III-V without secondary sensoria. Cornicles completely dark, without setae, slightly tapering to apical flange; approximately 3½-4½ times as long as wide, much longer than length of cauda. Cauda dark, stout, less than twice as long as wide with 2-3 pairs of lateral setae.

Winged adult female.- In life, body shape and coloration similar to wingless adult female; antennal segments I-VI dusky to dark on slide-mounted specimens; small to medium sized, body length 1.6-2.2 mm. Antennae 6 segmented; tubercles not developed; terminal process approximately 4⅓-5 times length of base of antennal segment VI; antennal segment III with 36-46 secondary sensoria; antennal segment IV with 13-21 secondary sensoria; antennal segment V with 2-4 secondary sensoria. Cornicles

completely dark, without setae, slightly tapering to apical flange; approximately 4¼-5½ times as long as wide, much longer than length of cauda. Cauda dark, stout, less than twice as long as wide with 3-4 pairs of lateral setae.

Hosts: Primary hosts include *Prunus* spp., especially *Prunus persica* (Blackman & Eastop 1984).

U.S. distribution: Widespread.

World distribution: Virtually worldwide.

Comments: *Brachycaudus persicae* is not listed as transmitting any plant viruses (Chan et al. 1991). Colonies of *B. persicae* occur on the roots of *Prunus* in late summer and during winter, however winter eggs are also laid (Blackman & Eastop 1984). In the spring, large colonies cluster around leaf buds and cause the death of young trees (Smith 1890b).

Brachycaudus schwartzi (Börner 1931)
Figs. 1, 6, 7

Synonymy:

* not listed

** *Brachycaudus schwartzi* (Börner 1931)

ESA approved common name: none

Other common names: peach aphid

Taxonomic characters: Wingless adult female.—In life, body color varying from dark brown to yellow brown with black dorsal abdominal patches. Small to medium sized, body length 1.7-2.2 mm, pear shaped. Antennae 6 segmented; tubercles not well developed; terminal process approximately 3-3⅓ times length of base of antennal segment VI; antennal segment III-V without secondary sensoria. Cornicles dark, without setae, with slight basal constriction then slightly tapering to apical flange; approximately 2-3 times as long as wide, longer than length of cauda. Cauda dark, stout, less than twice as long as wide with 2-3 pairs of lateral setae and 1-2 preapical setae.

Winged adult female.—In life, coloration similar to wingless adult female but body shape more elongate; antennal segments I-VI dusky on slide-mounted specimens; small to medium sized, body length 1.7-2.1 mm. Antennae 6 segmented; tubercles not well developed; terminal process approximately 3-3¾ times length of base of antennal segment VI; antennal segment III with 33-40 secondary sensoria; antennal segment IV with 4-13 secondary sensoria; antennal segment V without secondary sensoria. Cornicles dark, without setae, with slight basal constriction then slightly tapering to apical flange; approximately 1¾-2¾ times as long as wide, longer than length of cauda. Cauda dark, stout, less than twice as long as wide with 2-3 pairs of lateral setae and 1-2 preapical setae.

Hosts: Primary host is *Prunus persica* and occasionally *P. serotina* (Blackman & Eastop 1984).

U.S. distribution: California.

World distribution: Europe, Iran, India, South America, North America

Comments: *Brachycaudus schwartzi* is not listed as transmitting any plant viruses (Chan et al. 1991). Spring colonies can cause severe curling and disfiguration on peach leaves (Blackman & Eastop 1984).

Hyalopterus pruni (Geoffroy 1762)
Figs. 1, 4, 5

Synonymy:

* *Hyalopterus arundinis* (F. 1775)

** *Hyalopterus pruni* (Geoffroy 1762)

ESA approved common name: none

Other common names: mealy peach aphid, mealy plum aphid

Taxonomic characters: Wingless adult female.—In life body light green with darker green mottling, covered with waxy powder. Medium sized, body length 2.1-2.4 mm, body elongate. Antennae 6 segmented; tubercles not well developed; terminal process approximately 3 times length of base of antennal segment VI, antennal segment III-V without secondary sensoria. Cornicles dark, without setae, without apical flange, 2¼-2¾ times as long as wide, shorter than length of cauda. Cauda dark, elongate, nearly twice as long as wide with 2 pairs of lateral setae and 0-1 preapical seta.

Winged adult female.—In life abdomen green with wax patches on each segment; hind wing with 2 oblique veins; small sized, body length 1.7-1.9 mm. Antennae 6 segmented; tubercles not well developed; terminal process approximately 4 times length of base of antennal segment VI; antennal segment III with 18-27 secondary sensoria of variable size; antennal segment IV with 0-4 secondary sensoria; antennal segment V without secondary sensoria. Cornicles dark, without setae, 3-3⅓ times as long as wide, shorter than length of cauda. Cauda dark, elongate, nearly twice as long as wide with 2-3 pairs of lateral setae and 0-1 preapical seta.

Hosts: Primary hosts include several species of *Prunus* including *Prunus persica* (Blackman & Eastop 1984).

U.S. distribution: Widespread.

Distribution in the world: Virtually worldwide.

Comments: *Hyalopterus pruni* is very similar to *Hyalopterus amygdali* (Blanchard 1840) and the relationship between them remains unresolved. Basky & Szalay-Marszó (1987) found no reliable morphological differences between the two species. However, differences in host plant selection and mate choice led those authors to believe the two species were distinct. Blackman & Eastop (1994) summarized the literature pertaining to the disposition of *H. amygdali* and *H. pruni* and treated them as separate species.

Chan et al. (1991) recorded seven plant viruses transmitted by *H. pruni*, including plum pox virus. They did not list any plant viruses associated with *H. amygdali.*

Hysteroneura setariae (Thomas 1878)
Figs. 1, 4, 5

Synonymy:

* *Aphis setariae* (Thomas)

** *Hysteroneura setariae* (Thomas)

ESA approved common name: rusty plum aphid

Taxonomic characters: Wingless adult female.—In life body dark reddish brown, apical area of tibiae dark, cornicles dark to almost black, cauda pale to nearly white. Small to medium sized, 1.7-2.3 mm, body rounded. Antennae 6 segmented; tubercles not well developed, terminal process approximately 4⅔-5 times length of base of antennal segment VI; antennal segment III-V without secondary sensoria. Cornicles dark to nearly black, without setae, tapered apically, approximately 2¾-4⅔ times as long as wide, longer than length of cauda. Cauda pale to nearly white, elongate, more than twice as long as wide with 2-3 (usually 2) pairs of lateral setae.

Winged adult female.—In life coloration similar to wingless adult female; hind wing with one oblique vein; small to medium sized, body length 1.7-2.2 mm. Antennae 6 segmented; tubercles not well developed, terminal process approximately 5¼-7 times length of base of antennal segment VI; antennal segment III with 13-20 secondary sensoria of variable size; antennal segment IV with 1-5 secondary sensoria; an-

tennal segment V without secondary sensoria. Cornicles dark to nearly black, without setae, tapered apically, approximately 3¾-5⅓ times as long as wide, longer than length of cauda. Cauda pale to nearly white, elongate, more than twice as long as wide with 2 pairs of lateral setae.

Hosts: Primary host is usually *Prunus domestica* (Blackman & Eastop 1984); however, it also occurs on other species of *Prunus* including *P. persica*. Secondary hosts include numerous species of Gramineae.

U.S. distribution: Widespread.

Distribution in the world: Virtually worldwide.

Comments: *Hysteroneura setariae* transmits six plant viruses but is not listed as a vector of a peach virus (Chan et al. 1991).

Macrosiphum euphorbiae (Thomas 1878)
Figs. 1, 2, 3

Synonymy:
* *Macrosiphum solanifolii* (Ashmead 1882)
** *Macrosiphum euphorbiae* (Thomas)

ESA approved common name: potato aphid.

Other common names: none

Taxonomic characters: Wingless adult female.—In life, body usually of varying shades of green or pink. Medium to large sized, body length 2.2-3.8 mm, pear shaped. Antennae 6 segmented; tubercles well developed with inner faces divergent; terminal process approximately 4¼-6¼ times length of base of antennal segment VI; antennal segment III with 3-5 secondary sensoria on basal half; antennal segment IV-V without secondary sensoria; either entirely dark or only dark apically. Cornicles entirely pale or becoming increasingly dusky towards tip, without setae, with slight apical constriction and several rows of polygonal reticulations in constricted area, approximately 6-11⅓ times as long as wide, longer than length of cauda. Cauda pale, elongate, more than twice as long as wide with 8-10 lateral setae and 2-3 dorsal preapical setae.

Winged adult female.—In life, body usually of varying shades of green or pink; hind wing with 2 oblique veins; medium to large sized, body length 2.6-3.0 mm. Antennae 6 segmented; frontal tubercles well developed with inner faces divergent; terminal process approximately 5⅓-7⅓ times length of base of antennal segment VI; antennal segment III with 14-18 secondary sensoria on basal ¾; antennal segments IV-V without secondary sensoria; entirely dark except for segments I and II and base of III. Cornicles sometimes pale but usually progressively darker towards tip, without setae, with slight apical constriction and several rows of polygonal reticulations in constricted area, approximately 6⅔-13 times as long as wide, longer than length of cauda. Cauda pale, elongate, more than twice as long as wide with 8-10 lateral setae and 1-2 dorsal preapical setae.

Hosts: Primary hosts are several species of *Rosa*. Polyphagous and very damaging to many other host plants of economic importance.

U.S. distribution: Widespread.

World distribution: Virtually worldwide.

Comments: *Macrosiphum euphorbiae* transmits 67 plant viruses but is not listed as a vector of a peach virus (Chan et al. 1991).

Myzus cerasi (F.1775)
Figs. 1, 2, 3

Synonymy:
* & ** *Myzus cerasi* (F.)

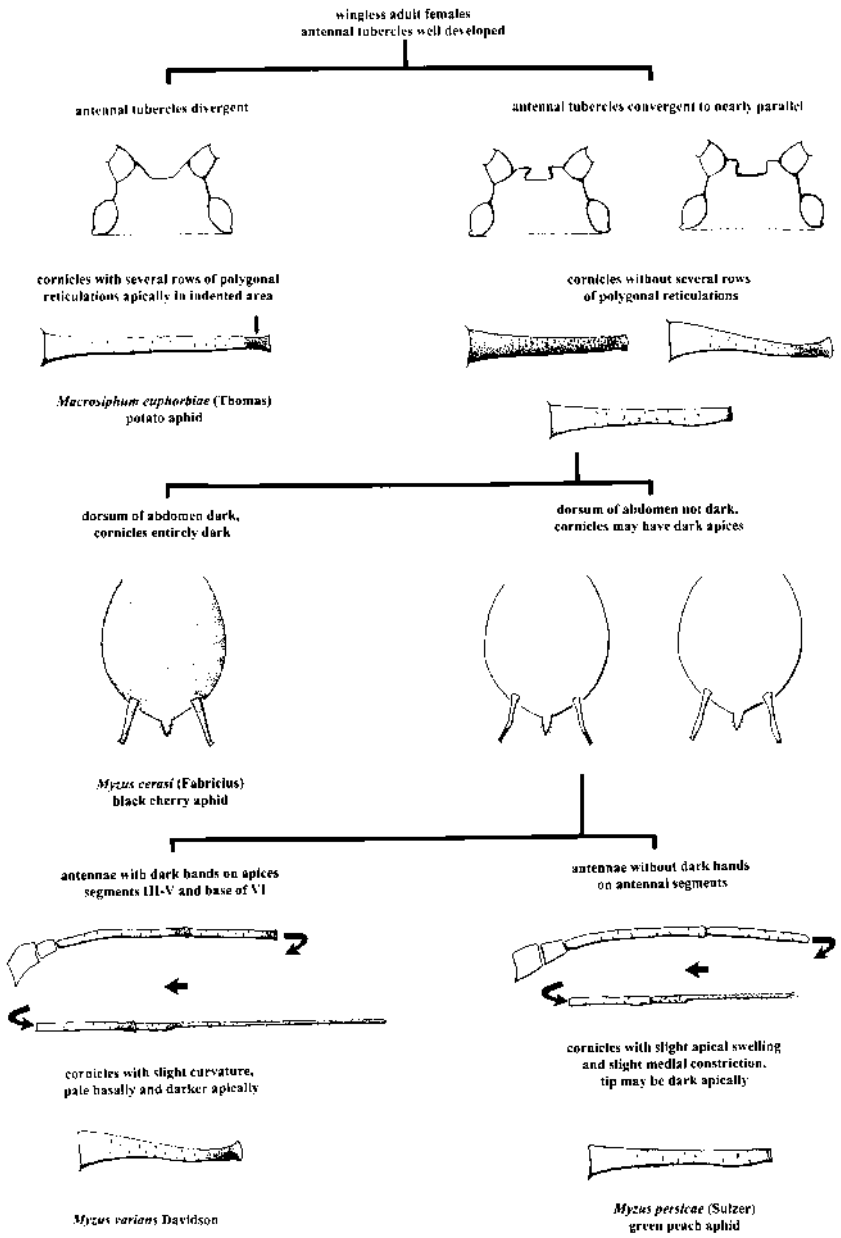

Fig. 2. Pictorial key to wingless adult females of four aphid species that potentially colonize on peaches in the United States and have well developed antennal tubercles.

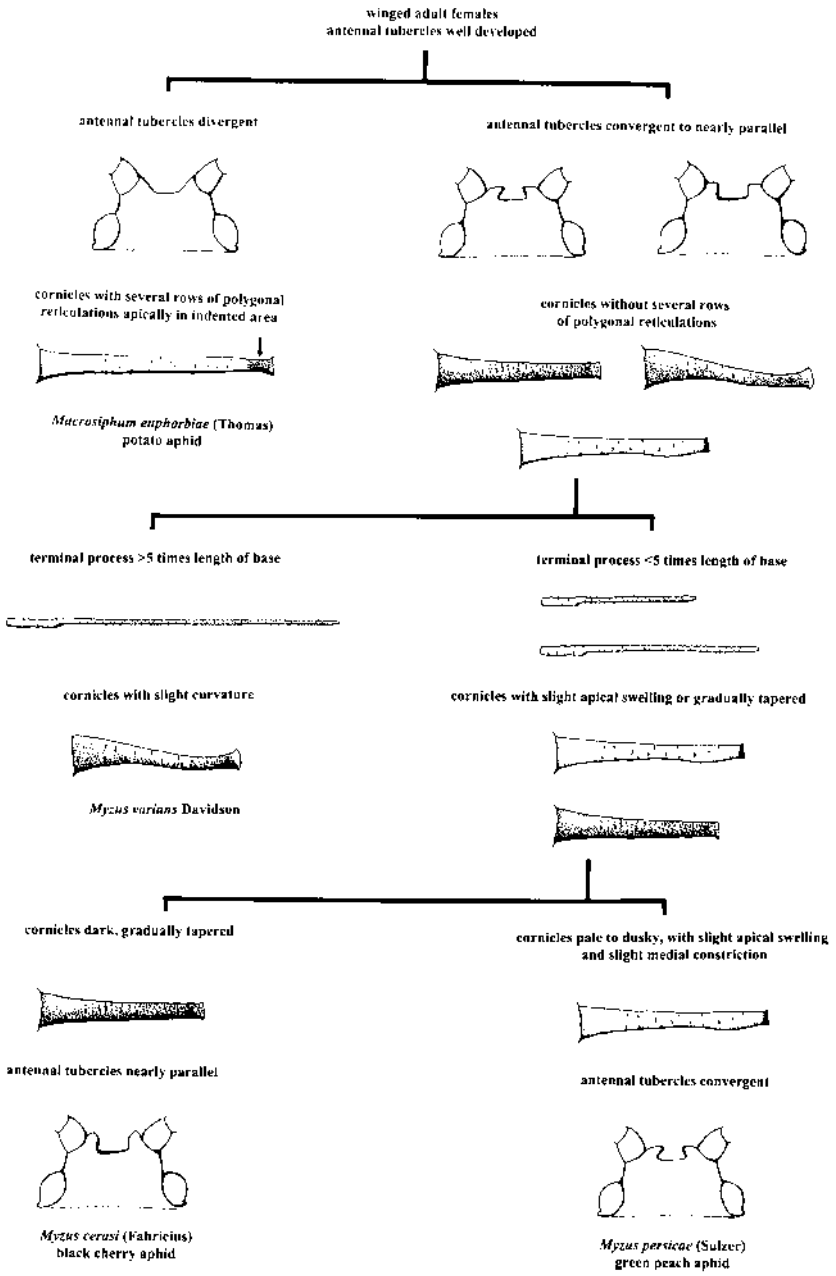

Fig. 3. Pictorial key to winged adult females of four aphid species that potentially colonize on peaches in the United States and have well developed antennal tubercles.

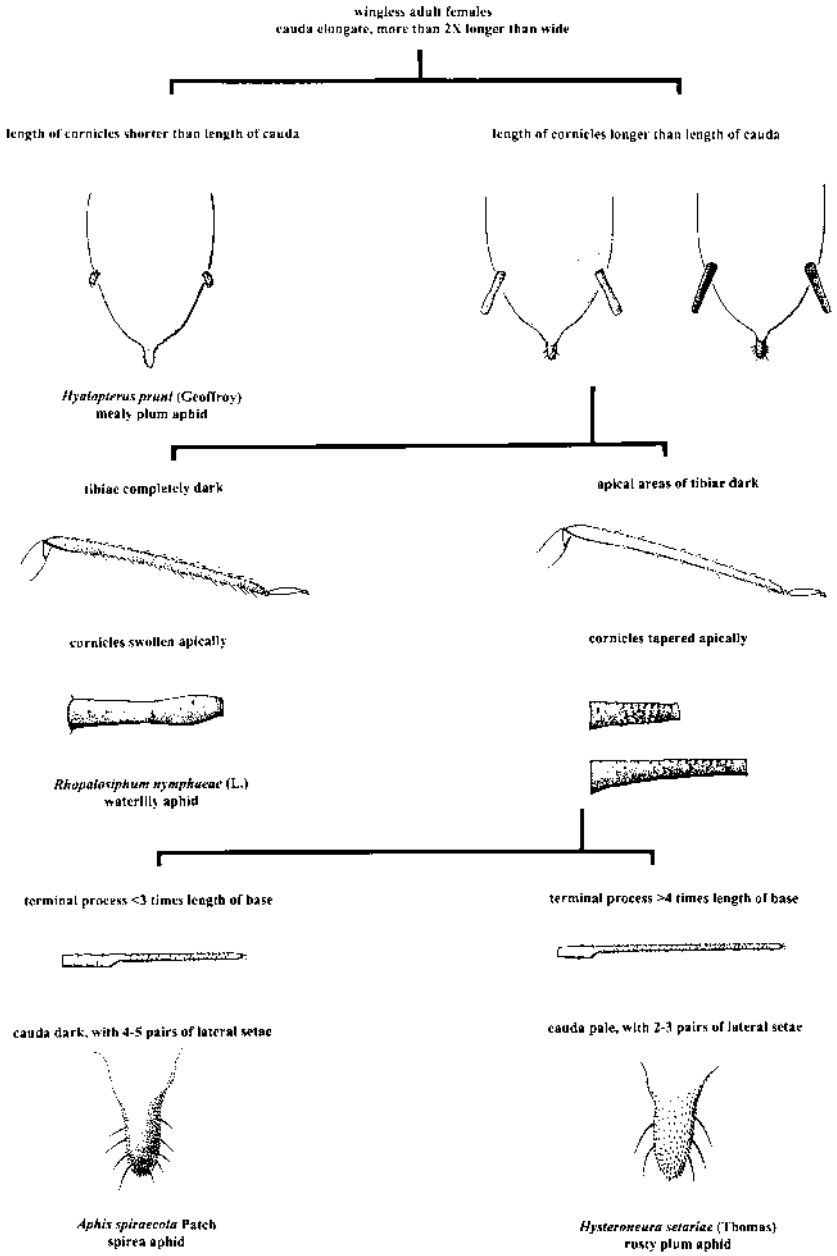

Fig. 4. Pictorial key to wingless adult females of four aphid species that potentially colonize on peaches in the United States with elongate cauda and antennal tubercles not developed.

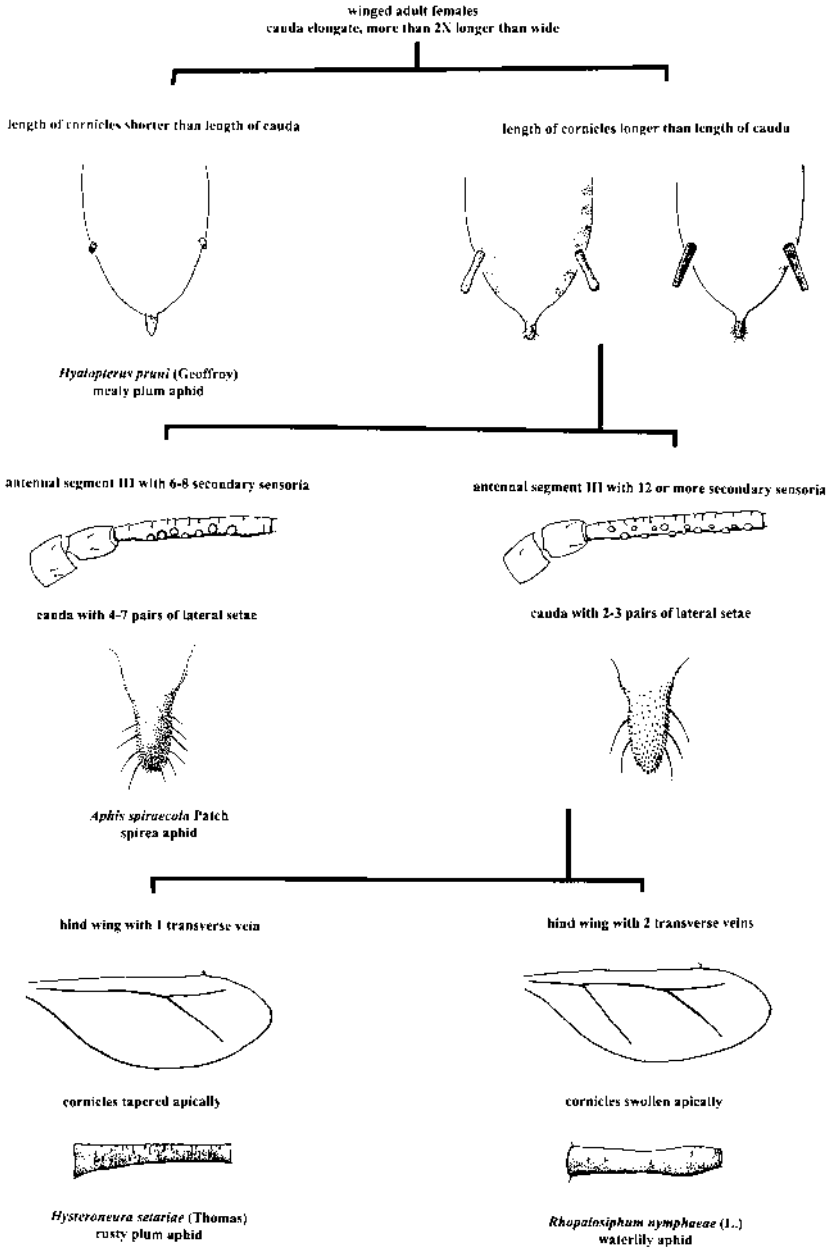

Fig. 5. Pictorial key to winged adult females of four aphid species that potentially colonize on peaches in the United States with elongate cauda and antennal tubercles not developed.

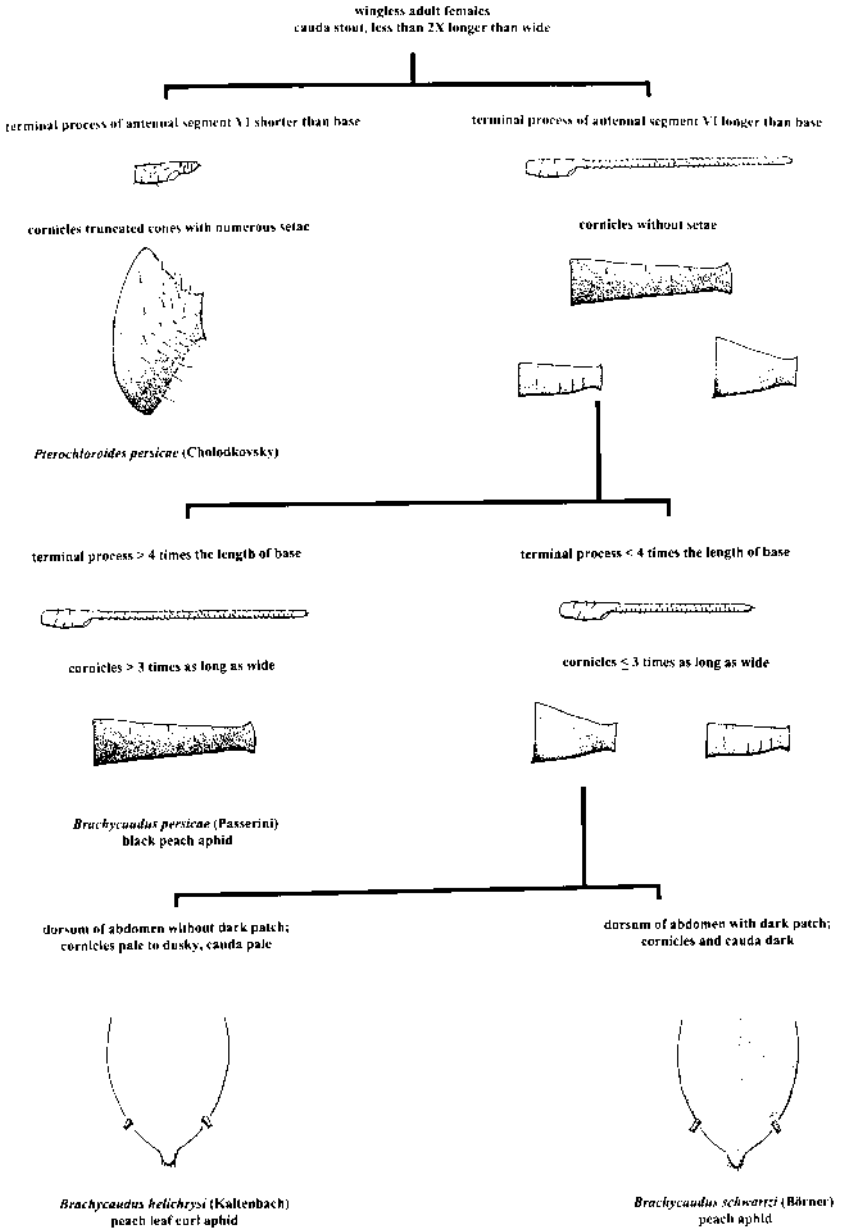

Fig. 6. Pictorial key to wingless adult females of four aphid species that potentially colonize on peaches in the United States with stout cauda and antennal tubercles not developed.

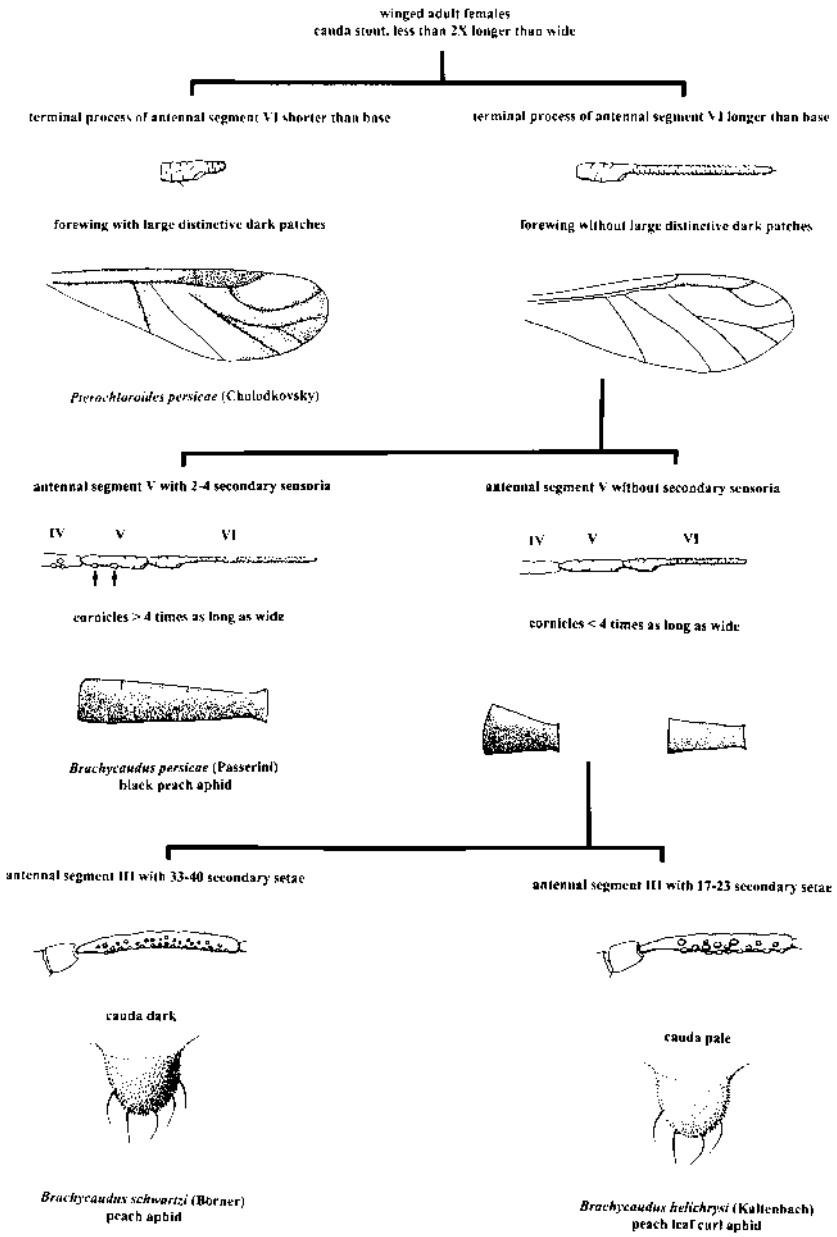

Fig. 7. Pictorial key to winged adult females of four aphid species that potentially colonize on peaches in the United States with stout cauda and antennal tubercles not developed.

ESA approved common name: black cherry aphid

Other common name: cherry blackfly

Taxonomic characters: Wingless adult female.—In life body color shiny dark brown to black with bicolored yellow and brown antennae and legs. Small to medium sized 1.9-2.2 mm, pear shaped. Antennae 6 segmented; tubercles well developed with inner faces nearly parallel, terminal process approximately 2-3¼ times length of base of antennal segment VI, antennal segment III-V without secondary sensoria. Cornicles dark, without setae, gradually tapered, approximately 5-9½ times as long as wide, longer than length of cauda. Cauda dark, elongate, more than twice as long as wide with 2-3 pairs of lateral setae.

Winged adult female.—In life body color with dorsal patch on abdomen; hind wing with 2 oblique veins; small sized, body length 1.7-1.9 mm. Antennae 6 segmented; tubercles well developed with inner faces nearly parallel, terminal process approximately 3-4 times length of base of antennal segment VI, antennal segment III with 12-16 secondary sensoria of similar size and in an irregular row; antennal segment IV with 0-3 secondary sensoria; antennal segment V without secondary sensoria. Cornicles dark, without setae, gradually tapered, approximately 5-6⅓ times as long as wide, longer than length of cauda. Cauda dark, elongate, more than twice as long as wide with 2-3 pairs of lateral setae.

Hosts: Primary hosts include *Prunus cerasus*, *P. avium* and occasionally other species of *Prunus*. Secondary hosts include species of Cruciferae, Rubiaceae, and Scrophulariaceae.

U.S. distribution: Widespread.

Distribution in the world: Australia, Europe, India, New Zealand, North America, Pakistan, Turkey.

Comments: *Myzus cerasi* transmits six plant viruses but is not listed as a vector of a peach virus (Chan et al. 1991).

Myzus persicae (Sulzer 1776)
Figs. 1, 2, 3

Synonymy:

* & ** *Myzus persicae* (Sulzer)

ESA approved common name: green peach aphid

Other common name: peach-potato aphid

Taxonomic characters: Wingless adult female.—In life, body varying from green to pale yellow. Small to medium sized, 1.9-2.4 mm, pear shaped. Antennae 6 segmented; tubercles well developed with inner faces convergent; terminal process approximately 2¾-3¾ times length of base of antennal segment VI; antennal segment III-V without secondary sensoria. Cornicles pale but tip may be dark, without setae, slight apical swelling and slight medial constriction; approximately 4¾-7 times as long as wide, longer than length of cauda. Cauda pale to dusky, elongate, more than twice as long as wide with 3 pairs of lateral setae.

Winged adult female.—In life, body varies from green to pale yellow with a large dark patch on dorsum of abdomen; hind wing with 2 oblique veins; small to medium sized, body length 1.7-2.3 mm. Antennae 6 segmented; tubercles well developed with inner faces convergent; terminal process approximately 3¼-4½ times length of base of antennal segment VI; 8-11 secondary sensoria of similar size in a straight row on antennal segment III; without secondary sensoria on antennal segments IV-V. Cornicles dusky to dark but tip sometimes darker, without setae, slight apical swelling and slight medial constriction; approximately 3¾-6 times as long as wide, longer than

length of cauda. Cauda pale to dusky, elongate, more than twice as long as wide with 3 pairs of lateral setae.

Hosts: Primary hosts are several species of *Prunus*. Polyphagous and very damaging to many other host plants of economic importance.

U.S. distribution: Widespread.

World distribution: Virtually worldwide.

Comments: *Myzus persicae* transmits 182 plant viruses including plum pox virus (Chan et al. 1991).

Myzus varians Davidson 1912
Figs. 1, 2, 3

Synonymy:
* not listed
** *Myzus varians* Davidson

ESA approved common name: none

Other common name: none

Taxonomic characters: Wingless adult female.—In life, body varying from light to darker green. Small sized, body length 1.6-1.9 mm, pear shaped. Antennae 6 segmented with dark bands on apices antennal segments III, IV, V, and base of VI; tubercles well developed with inner faces convergent; terminal process approximately 5¼-5½ times length of base of antennal segment VI; antennal segment III-V without secondary sensoria. Cornicles pale basally and dark apically, without setae, slightly tapered with slight curvature; 4-5⅓ times as long as wide, longer than length of cauda. Cauda pale, elongate, more than twice as long as wide with 3-4 pairs of lateral setae.

Winged adult female.—In life, body varies from green to blue-green with a dark patch on the dorsum of abdomen; hind wing with 2 oblique veins; small to medium sized, body length 1.7-2.2 mm. Antennae 6 segmented, completely dark; tubercles well developed with inner faces convergent; terminal process approximately 5¼-5⅔ times length of base of antennal segment VI; antennal segment III with 5-12 secondary sensoria of similar size in a straight row; antennal segments IV-V without secondary sensoria. Cornicles dark, without setae, slightly tapered with slight curvature; 4⅓-7 times as long as wide, longer than length of cauda. Cauda pale, elongate, more than twice as long as wide with 3-5 pairs of lateral setae.

Hosts: Primary host is *Prunus persica* in spring and the secondary host is *Clematis* spp. in the summer. However, in North America *M. varians* has been collected predominately on *Clematis* spp.

U.S. distribution: California, Florida, Maryland, North Carolina

World distribution: Europe, East Asia, North America

Comments: Although not listed in Chan et al. (1991), *M. varians* has been recorded as transmitting plum pox virus (Blackman & Eastop 1984).

Pterochloroides persicae (Cholodkovsky 1899)
Figs. 1, 6, 7

Synonymy:
* not listed
** *Pterochloroides persicae* (Cholodkovsky)

ESA approved common name: none

Other common name: clouded peach bark aphid, cloudy-winged peach aphid

Taxonomic characters: Wingless adult female.—In life body color dark brown to black with some white patches, dorsum of abdomen with a double row of large tubercles; venter white. Large sized, body length 3.5-4.7 mm, oval. Antennae 6 segmented,

short with apical bands on antennal segments III-VI; tubercles not well developed; terminal process shorter than length of base antennal segment VI; antennal segment III with 0-2 secondary sensoria; 0-1 secondary sensoria on antennal segment IV; antennal segment V without secondary sensoria. Cornicles dark, truncated cones with numerous setae, nearly as long as wide, subequal to length of cauda. Cauda dark, stout, less than 2× longer than wide with numerous setae.

Winged adult female.—In life body color similar to wingless adult female; forewing with large distinctive dark patches; large sized, body length 2.7-3.6 mm. Antennae 6 segmented, short, terminal process less than length of base antennal segment VI; antennal segment III with 8-14 secondary sensoria of similar size; antennal segment IV with 1-5 secondary sensoria; antennal segment V without secondary sensoria. Cornicles dark, truncated cones with numerous setae, nearly as long as wide, subequal to length of cauda. Cauda dusky, stout, less than 2× longer than wide with numerous setae.

Hosts: Principle hosts include *Prunus* spp. (almond, apricot, peach), however *P. persicae* has also been recorded from other plants including *Citrus* and *Malus*. *Pterochloroides persicae* is found living on large branches and trunks of its host (Blackman & Eastop 1994).

U.S. distribution: Not known to occur in the United States.

Distribution in the world: Recorded from India, Pakistan, the Middle East, the Mediterranean area, Italy and Yugoslavia.

Comments: Although *P. persicae* has not been recorded from the United States, it remains a potential pest because of its range of economically important hosts (Stoetzel 1990). Large populations of *P. persicae* occurring on the bark can cause fruit not to develop or premature fruit drop; this species produces large amounts of honeydew and is tended by ants (Stoetzel 1994). *Pterochloroides persicae* is not listed as transmitting a virus (Chan et al. 1991).

Rhopalosiphum nymphaeae (L. 1761)
Figs. 1, 4, 5

Synonymy:

* & ** *Rhopalosiphum nymphaeae* (L.)

ESA approved common name: waterlily aphid

Taxonomic characters: Wingless adult female.—In life body color olive green to golden brown, with waxy covering on head and prothorax; tibiae completely dark. Medium sized, body length 2.0-2.5 mm, rounded. Antennae 6 segmented; tubercles not well developed, terminal process approximately 3-3¾ times length of base antennal segment VI; antennal segment III-V without secondary sensoria. Cornicles dusky, without setae, swollen with apical constriction proximal to flange, 2⅔-5 times as long as wide, longer than length of cauda. Cauda dusky, elongate, nearly twice as long as wide with 2-3 pairs of lateral setae.

Winged adult female.—In life body color similar to wingless adult female; hind wing with 2 oblique veins; small to medium sized, body length 1.4-2.5 mm. Antennae 6 segmented, terminal process approximately 3-3¾ times length of base antennal segment VI; antennal segment III with 12-22 secondary sensoria of similar size; antennal segment IV with 0-5 secondary sensoria; antennal segment V without secondary sensoria. Cornicles dusky, without setae, swollen with apical constriction proximal to flange, 4¾-7 times as long as wide, longer than length of cauda. Cauda dusky, elongate, nearly twice as long as wide with 2 pairs of lateral setae.

Hosts: In the spring, *R. nymphaeae* feeds on the young twigs, leaf petioles, and fruit stalks of numerous species of *Prunus*; various species of aquatic plants serve as secondary hosts.

U.S. distribution: Widespread.

Distribution in the world: Virtually worldwide.

Comments: *Rhopalosiphum nymphaeae* transmits six plant viruses, but is not listed as a vector of a peach virus (Chan et al. 1991).

KEY TO THE WINGLESS ADULT FEMALES OF APHID SPECIES POTENTIALLY COLONIZING ON PEACH IN THE UNITED STATES

1. Terminal process of antennal segment VI shorter than base; cornicles truncated cones with numerous setae . *Pterochloroides persicae* (Cholodkovsky)
   Terminal process of antennal segment VI longer than base; cornicles various but without setae 2
2. Cornicles distinctly shorter than length of cauda . . . . . *Hyalopterus pruni* (Geoffroy), mealy plum aphid
   Cornicles subequal or longer than length of cauda 3
3(2). Antennal tubercles not well developed, not extending beyond frons or approximately even with frons (Fig. 1) 7
   Antennal tubercles well developed, extending beyond frons (Fig. 1) 4
4(3). Cornicles with apical region of polygonal reticulation . . . . . *Macrosiphum euphorbiae* (Thomas), potato aphid
   Cornicles without apical region of polygonal reticulation 5
5(4). Cornicles entirely dark; abdomen with large dorsal patch . . . . . *Myzus cerasi* (F.), black cherry aphid
   Cornicles pale, tips may be dark; abdomen without large dorsal patch . . . 6
6(5). Terminal process >5 times length of base; cornicles constricted medially, slightly swollen apically . . . . . *Myzus persicae* (Sulzer), green peach aphid
   Terminal process <5 times length of base; cornicles slightly tapered with slight curvature . . . . . *Myzus varians* Davidson
7(3). Cauda stout, less than 2× longer than wide 8
   Cauda elongate, nearly 2× or more longer than wide 10
8(7). Terminal process >4 times length of base; cornicles >3 times as long as wide . . . . . *Brachycaudus persicae* (Passerini), black peach aphid
   Terminal process <4 times length of base; cornicles 3 times as long as wide 9
9(8). Abdomen without dark dorsal markings; cauda pale . . . . . *Brachycaudus helichrysi* (Kaltenbach), peach leaf curl aphid
   Abdomen with dark dorsal markings; cauda dark . . . . . *Brachycaudus schwartzi* (Börner), peach aphid
10(7). Terminal process >4 times length of base; cornicles dark and cauda pale . . . . . *Hysteroneura setariae* (Thomas), rusty plum aphid
   Terminal process <4 times length of base; cornicles dark or dusky and cauda dark 11
11(10). Cornicles swollen apically; cauda with 2-3 pairs of lateral setae . . . . . *Rhopalosiphum nymphaeae* (L.), waterlily aphid
   Cornicles gradually tapered; cauda with 3-5 pairs of lateral setae . . . . . *Aphis spiraecola* Patch, spirea aphid

KEY TO THE WINGED ADULT FEMALES OF APHID SPECIES POTENTIALLY COLONIZING ON PEACH IN THE UNITED STATES

1. Forewing with large distinctive dark patches; terminal process of antennal segment VI shorter than base . . . . *Pterochloroides persicae* (Cholodkovsky)

Forewing without large distinctive dark patches; terminal process of antennal segment VI longer than base 2

2. Cornicles distinctly shorter than length of cauda . . . . . *Hyalopterus pruni* (Geoffroy), mealy plum aphid

Cornicles subequal or longer than length of cauda 3

3(2). Antennal tubercles not well developed, not extending beyond frons or approximately even with frons 7

Antennal tubercles well developed, extending beyond frons 4

4(3). Cornicles with apical region of polygonal reticulation . . . . . *Macrosiphum euphorbiae* (Thomas), potato aphid

Cornicles without apical region of polygonal reticulation 5

5(4). Terminal process >5 times length of base; cornicles with slight curvature . . . . . *Myzus varians* Davidson

Terminal process <5 times length of base; cornicles tapered slightly or with slight medial constriction, without curvature 6

6(5). Antennal segment III with 8-11 secondary sensoria of similar size and in a straight row; cornicles with slight medial constriction . . . . . *Myzus persicae* (Sulzer), green peach aphid

Antennal segment III with 12-16 secondary sensoria of similar size and in an irregular row; cornicles tapered slightly and curved . . . . . *Myzus cerasi* (F.), black cherry aphid

7(3). Cauda stout, less than 2× longer than wide 8

Cauda elongate, nearly 2× or more longer than wide 10

8(7). Terminal process >4 times length of base; antennal segment V with 2-4 secondary sensoria . . . . *Brachycaudus persicae* (Passerini), black peach aphid

Terminal process <4 times length of base; antennal segment V without secondary sensoria 9

9(8). Antennal segment III with 17-23 secondary sensoria; cornicles dusky and cauda pale . . . . . *Brachycaudus helichrysi* (Kaltenbach), peach leaf curl aphid

Antennal segment III with 33-40 secondary sensoria; cornicles and cauda dark . . . . . *Brachycaudus schwartzi* (Börner), peach aphid

10(7). Terminal process >5 times length of base; cornicles dark and caudal pale to nearly white . . . . . *Hysteroneura setariae* (Thomas), rusty plum aphid

Terminal process <4 times length of base; cornicles dark or dusky and cauda dark 11

11(10). Cornicles swollen apically; cauda with 2-3 pairs of lateral setae . . . . . *Rhopalosiphum nymphaeae* (L.), waterlily aphid

Cornicles gradually tapered; cauda with 3-7 pairs of lateral setae . . . . . *Aphis spiraecola* Patch, spirea aphid

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DEVELOPMENT AND FECUNDITY OF *DERAEOCORIS NEBULOSUS* (HETEROPTERA: MIRIDAE) ON *BEMISIA ARGENTIFOLII* (HOMOPTERA: ALEYRODIDAE)

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ABSTRACT

The developmental and reproductive biology of the native predaceous mirid *Deraeocoris nebulosus* was studied in the laboratory using immatures of the whitefly *Bemisia argentifolii* as prey. Nymphs were kept individually in ventilated Petri dishes and provided with a constant supply of prey colonized on excised sweet potato leaves rooted in hydroponic solution and kept at 27°C. Females were kept similarly and daily egg production was recorded. There were five nymphal instars. Mean development from first instar to adult was 13.3 d; there were no significant differences in development rate between the sexes. After a 3-d preoviposition period, females produced about 10-14 eggs per day for nearly 20 days before oviposition rate declined with age. Females lived an average of 32.8 d (range 3-58 d), and mean fecundity was 242.3 eggs per female (range 0-392).

Key Words: predator, whitefly, biological control, biology, rearing

RESUMEN

La biología reproductiva y el desarrollo del mírido depredador nativo *Deraecoris nebulosus* se estudiaron en condiciones de laboratorio utilizando ninfas de la mosquita blanca, *Bemisia argentifolii,* como presas. Las ninfas del mírido se mantuvieron individualmente en cajas de Petri ventiladas y a una temperatura constante de 27°C. Las presas fueron constantemente presentadas a los depredadores en hojas de camote enraizadas en tubos de plástico conteniendo una solución hidropónica. Las hembras de los depredadores se mantuvieron en condiciones ambientales similares a las de las ninfas y la producción de huevecillos se registró diariamente. Se detectaron 5 estadíos

ninfales. El promedio del tiempo de desarrollo del primer estadío al adulto fue de 13.3 días; no se detectaron diferencias significativas en el tiempo de desarrollo entre machos y hembras. Después de un período pre-oviposicional de 3 días las hembras produjeron diariamente de 10 a 14 huevecillos por casi 20 días continuos, disminuyendo después debido a la edad. El promedio de longevidad de las hembras fue de 32.8 días con una fluctuación de 3 a 58 días y el promedio de fecundidad por hembra fue de 242.3 huevecillos con una fluctuación de 0 a 392 huevecillos.

The dramatic increase in the economic importance of the *Bemisia tabaci* (Gennadius) species complex has been attributed to the virtual replacement of the sweetpotato whitefly, *B. tabaci* (= biotype A), with a new species, the silverleaf whitefly, *B. argentifolii* Bellows & Perring (= sweetpotato whitefly *B. tabaci*, biotype B). The appearance of this new pest has generated widespread activity aimed at developing management methods that minimize additional pesticide load in the environment. Manipulative biological control methods are being investigated for application in greenhouse and field crops. Certain predaceous Miridae might have potential for managing pest whiteflies, particularly in affected greenhouse crops (e.g. Malausa et al. 1987, Alomar et al. 1990, Fransen 1994). Research has been conducted in Europe on various biological aspects of predaceous mirids in the genera *Cyrtopeltis*, *Dicyphus,* and *Macrolophus* (e.g. Fauvel et al. 1987, Malausa 1989, Fransen 1994). *Macrolophus caliginosus* Wagner is currently sold commercially for whitefly control (van Schelt et al. 1996. Hunter 1997). *Deraeocoris* spp. have also been recognized as efficient predators of whiteflies, and their potential has recently been evaluated against *B. tabaci* (Susman 1988, Kapadia & Puri 1991). The North American species *D. brevis* (Uhler) is sold commercially for whitefly management (Hunter 1997).

Deraeocoris nebulosus (Uhler) occurs throughout most of the United States and Canada (Carvalho 1957, Henry & Wheeler 1988). Its value as a predator was recognized over a century ago (Uhler 1876, Howard 1895). Field populations of *D. nebulosus* can be high. This predator was observed in commercial cotton fields in association with aphids in west-central Mississippi, even under heavy insecticide use (Snodgrass 1991) and has been associated with whitefly infestations in cotton there in recent years (G.L.S., unpublished). Aspects of the biology of *D. nebulosus* have been studied previously with the oak lace bug *Corythucha arcuata* (Say) (Wheeler et al. 1975) and the cotton aphid *Aphis gossypii* Glover (Snodgrass 1991) as prey. Wheeler et al. (1975) critically reviewed the literature concerning *D. nebulosus* and summarized the various host and habitat associations of this well-known predator; whiteflies (Aleyrodidae) and other sessile Homoptera are prominently mentioned. The goals of the present study were to determine if *B. argentifolii* is a suitable prey for development and reproduction of *D. nebulosus*, and to provide basic information for further investigations on the potential of this predator as a management tool against the *Bemisia* spp. complex and other whiteflies.

Materials and Methods

Insects were colonized from several dozen nymphs and adults collected in cotton near Stoneville, Washington County, in west-central Mississippi in August 1996. The duration of each immature stage was measured on F_1 progeny from the field-collected insects. Fecundity was derived for females from the development rate observations. To obtain eggs, about 10 unsexed adults from the initial field collection were placed to-

gether in each of several 120mm × 25 mm ventilated plastic culture dishes. Each dish contained a whitefly-infested sweet potato leaf. Leaves had previously been excised and placed individually in floral aquapics where they readily rooted in hydroponic solution (Aqua-Ponics International, Los Angeles, CA 90041). Prior observations indicated that eggs are deposited primarily in the leaf petioles and main leaf veins. First instar nymphs were obtained on the day of eclosion by daily examination of each leaf assembly containing eggs. Because preliminary observations also suggested that nymphs sometimes prey on each other when confined, these studies used isolated individuals. Thirty, newly emerged, nymphs were placed individually in a 120mm × 25 mm ventilated plastic culture dish with a rooted sweet potato leaf containing about 250 *B. argentifolii* nymphs of various ages. All tests were conducted at 27 ± 2°C, 55 ± 10% RH, and a photoperiod of 16:8 (L:D). Test insects were examined daily for change to the next instar or stage. Leaves with host nymphs were changed every 3-4 days. The sex of each insect was determined when it reached the adult stage.

Fecundity was measured by placing individual, teneral females with one or two males of mixed age, in dishes with infested leaves as described above. Males were not replaced after death. Leaves were examined daily for eggs. Eggs deposited over each 24-hr period were marked with colored ink so that one day's egg production could be distinguished from the next. Viability was not determined. Leaves were kept with each female for 3-4 days before replacement. A continuous series of infested leaves was kept with each female until her death. Some qualitative behavioral observations on mating, oviposition, and foraging were also made.

Developmental data were analyzed by ANOVA, and compared by sex using a t-test. Mean developmental time per instar and sex were subjected to Tukey's HSD test ($P < 0.05$) (SYSTAT, Inc. 1992).

Results and Discussion

Eggs were usually found embedded in plant tissue with only the long hairlike micropylar process protruding as described by McCaffrey & Horsburgh (1980); the cap was usually visible through the oviposition slit. Occasionally, eggs protruded from the plant surface or were not embedded at all. Unembedded eggs were not observed for eclosion. Although not specifically recorded, eggs sometimes were observed to be deposited in small groups. Generally, more eggs were deposited in the leaf petiole than in the leaf veins. McCaffrey & Horsburgh (1980) previously reported that eggs of *D. nebulosus* were deposited in leaf mid-veins of apple, but not in petioles or twigs. Other predaceous mirids have been reported to deposit their eggs in major leaf veins and leaf petioles (Cobben 1968, Khristova et al. 1975, Ferran et al. 1996).

There were five nymphal stages, as previously reported by Wheeler et al. (1975). Kapadia & Puri (1991) reported that a *Deraeocoris* sp. in India had six nymphal stages. Twenty-four of 30 nymphs reached adult, 16 males and only 8 females; at least two deaths were due to injury during handling. Total time from egg eclosion to adult at 27°C averaged 13.3 d (Table 1). There was no significant difference in development rate by sex (df = 22; $P = 0.69$). Wheeler et al. (1975) reported development of *D. nebulosus* to take 19.8-d at 21-22°C; Westigard (1973) recorded *D. brevis piceatus* Knight to have a mean development time of 25 days at 21°C. Susman (1988) reported the nymphal duration of *D. pallens* Reuter to be 11.1 days at 25-28°C. Differences among reported development rates is at least partially a function of the different temperatures and species used.

Mean egg deposition per female was 1.5 on day four, then ranged between 9.5-13.9 eggs per day until day 22, whereupon egg production began to decrease with increasing age (Fig. 1). Daily egg production was calculated on the basis of the number of fe-

TABLE 1. DEVELOPMENT TIME FOR EACH INSTAR OF *DERAEOCORIS NEBULOSUS* NYMPHS FED NYMPHS OF *BEMISIA ARGENTIFOLII*.

	Mean Development time (days ± SE)		
Instar	Male	Female	Male + Female
I	2.5 ± 0.18	2.8 ± 0.25	2.6 ± 0.15
II	2.0 ± 0.12	2.0 ± 0.19	2.0 ± 0.10
III	2.3 ± 0.14	2.1 ± 0.35	2.2 ± 0.15
IV	2.2 ± 0.10	2.8 ± 0.25	2.4 ± 0.12
V	4.4 ± 0.24	3.5 ± 0.19	4.1 ± 0.19
Total	13.3 ± 0.30	13.1 ± 0.30	13.3 ± 0.22

males surviving for a given day. One individual lived 58 days, depositing its last eggs at day 57. Mean fecundity was 242.3 eggs per female, ranging from 0-392. No *D. nebulosus* deposited eggs before the third day as an adult. Fecundity and female longevity were greater than that previously reported for any other predaceous mirid. Fecundity in *D. nebulosus* has not previously been reported. The oviposition period of an Indian species of *Deraeocoris* fed with *B. tabaci* averaged 11.3 days, with females living an average of 13.4 days at about 24°C (Kapadia & Puri 1991); *D. pallens* females deposited 23-268 eggs per female over an average lifespan of 14-34 days when fed with *B.*

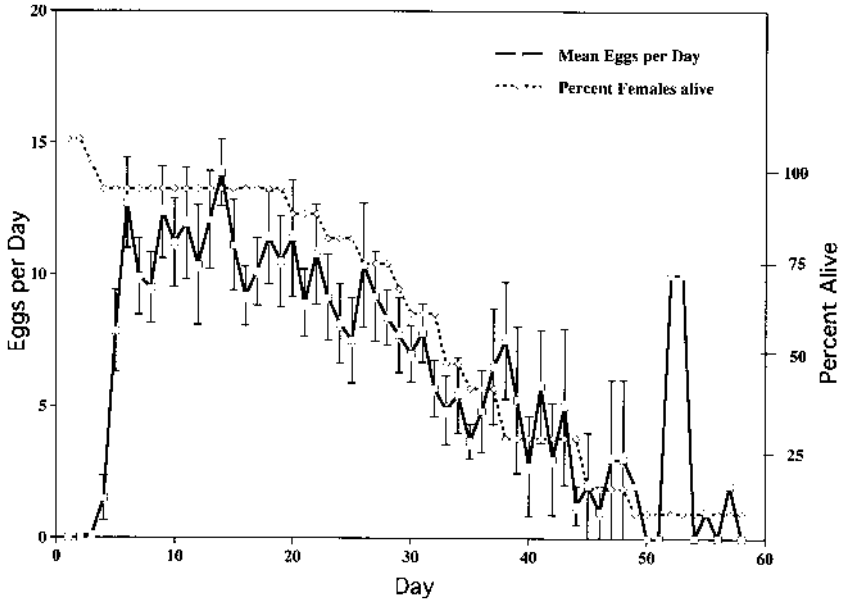

Fig. 1. Mean daily egg production per female (solid line), and daily survival (dotted line) of female *D. nebulosus*.

tabaci (Susman 1988). The commercially available predator *M. caliginosus*, when fed whitefly immatures, had a preoviposition period of 4 days at 23°C, and began oviposition at two eggs per day, which increased to over seven eggs per day for the rest of a 15-d study on fecundity (van Schelt et al. 1996).

Qualitative observations suggested that most foraging may not take place during daytime hours. Nymphs and especially adults were usually found resting under leaves or under the filter paper. When maintained in the greenhouse, these insects were primarily found within leaf litter of potted plants. Nevertheless, it was difficult keeping enough whitefly immatures to maintain the colony. Mating and oviposition was rarely witnessed.

Our preliminary observations showed that nymphs probably prey on each other when confined. Thus, the possibility of production for release against whiteflies may require special rearing conditions that minimize cannibalism.

These results demonstrate that *D. nebulosus* can survive, develop, and reproduce normally using *B. argentifolii* immatures as prey, and that the fecundity of this predator is greater than that of other predaceous mirids previously tested on any host. There is no evidence that this species is also partially phytophagous, as is the case with certain other predaceous mirids studied for their potential in managing whiteflies. Further studies are warranted to measure the efficacy of *D. nebulosus* against *Bemisia* spp., as well as other pests that are potential prey of this mirid.

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MATING BEHAVIOR AND SEXUAL RESPONSE TO AGGREGATION PHEROMONE OF *RHYNCHOPHORUS CRUENTATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Mating behavior of *Rhynchophorus cruentatus* (Fabricius) was investigated in the laboratory. The sequence of behaviors was consistent for all weevils that mated. Males exhibited rostral rubbing and antennal tapping before copulation, guarding females afterwards. Mating frequency and celerity were compared between sequestered male-female pairs and for focal males in simulated aggregations. Field-collected and virgin males in groups were significantly quicker to initiate mating behavior and attempted to mate more often per session than males in sequestered pairs. Increased sexual stimulation in weevil aggregations appears to be semiochemically-mediated. Visual and tactile cues were eliminated as contributing stimuli in simulated aggregation arenas. Males were significantly more stimulated to mate in the presence of synthetic aggregation pheromone, 5-methyl-4-octanol (cruentol) than in its absence.

Key words: aggregation, mating behavior, pheromone, *Rhynchophorus cruentatus,* 5-methyl-4-octanol

RESUMEN

El comportamiento de apareo de *Rhynchophorus cruentatus* (Fabricius) fue investigado en el laboratorio. La secuencia de comportamientos fue la misma para todos los gorgojos que se aparearon. Los machos exhibieron frotamiento con el rostro y golpecitos con las antenas antes de la copulación, la que fue seguida por la guardia de las hembras. La frecuencia y rapidez del apareo fueron comparados entre parejas aisladas de macho y hembra y machos focales en agregaciones artificiales. Se observó que los machos colectados en el campo y los machos vírgenes en agregaciones iniciaban el comportamiento de apareo significativamente más rápido y que intentaban aparearse más seguido en cada sesión que los machos de parejas aisladas. El incremento de la estimulación sexual en agregaciones de gorgojos parece estar mediado por un compuesto semioquímico. Fueron eliminados los estímulos visuales y tactiles como contribuyentes al apareo dentro de arenas con agregaciones artificiales de gorgojos. Los machos fueron significativamente más estimulados para aparearse en la presencia de la feromona sintética de agregación 5-metil-4-octanol (cruentol) que en su ausencia.

The palmetto weevil, *Rhynchophorus cruentatus* (F.), is the largest weevil (24-33 mm long) in the continental United States (Woodruff 1967). This weevil is sympatric with the native cabbage palmetto, *Sabal palmetto* (Walter) (Woodruff 1967) from the Florida Keys through the coastal regions of South Carolina and Texas (Wattanapongsiri 1966). In most cases, *R. cruentatus* is not considered to be a primary pest of cabbage palmetto, but a secondary pest that attacks transplanted or otherwise stressed

palms in the landscape (Giblin-Davis & Howard 1989). Recent research suggests that it can be a serious pest of field-grown *Phoenix canariensis* (L.) (unpublished data). The weevil is attracted to the fermenting plant volatiles from chopped palm tissue and chopped sugarcane, *Saccharum officinarum* (Weissling et al. 1993). There is a synergistic effect when these plant volatiles are combined with *S,S*-5-methyl-4-octanol (cruentol), the aggregation pheromone produced by *R. cruentatus* males (Weissling et al. 1994a; Perez et al. 1994). Large aggregations of male and female *R. cruentatus* are attracted to this combination, presumably for mating and oviposition purposes (Giblin-Davis et al. 1994; Weissling et al. 1993).

Many species of insects and other animals are known to aggregate for mating purposes (Landolt 1997). Evolutionary biologists have investigated advantages for males calling in conspecifics (which invites competition from other males) (Thornhill & Alcock 1984) and the advantages of multiple mating for females (Sakurai 1996; Lewin 1988). The production of aggregation pheromones may serve to recruit widely distributed conspecifics to rare resources (i.e. stressed hosts). Therefore, it may be advantageous to males to call females as a way to reduce the amount of time spent searching for widely dispersed potential mates. This may also be an adaptation that enables *R. cruentatus* to cope with the seasonal emergence patterns characteristic of this species (Weissling et al. 1994b), and to overwhelm the defenses of a potential palm host (Giblin-Davis et al. 1996).

Rhynchophorus cruentatus males are morphologically well adapted to mating in aggregations. On the tibiae of the forelegs, males possess a row of setae, commonly referred to as a "sex comb". A similar structure is used by males of some other species of insects to facilitate grasping and control of females (Spieth 1952). This structure may also prevent aggregated males from dislodging copulating males. Another possible function for the tibial hairs may be to distribute pheromone. Males of *R. palmarum* (L.) apparently produce pheromone in the prothoraxic glands and pass the molecules forward to the rostrum where it is distributed by setae (Sanchez et al. 1996). *Rhynchophorus cruentatus* is the only species of *Rhynchophorus* that lacks these rostral setae but instead possesses rostral ferrugae (Wattanapongsiri 1966). Possibly they wipe the rostrum with the tibial hairs to help distribute pheromone. Because little is known about the mating behavior of the agriculturally important Rhynchophorinae we endeavored to characterize the mating behavior of *R. cruentatus* in the laboratory.

Materials and Methods

Adult weevils were collected in Hendry and Dade Counties, Florida during the peak trapping seasons of 4/96-7/96 and 4/97-7/97. In Hendry County, 120 weevils were collected in four live traps set in a field with cabbage palmettos from which the buds had been removed by "palm heart" collectors. In Dade County, weevils were trapped from a grove of *P. canariensis* that had sustained severe damage from *R. cruentatus*. Fifty-five virgin weevils (harvested directly from cocoons) and 140 adults with unknown mating histories were collected. The trap design used was previously described by Weissling et al. (1992). Each trap was baited with sugarcane (400 g), ethyl acetate (6 ml) in open-topped vials and one controlled-release dispersal unit containing synthetic racemic cruentol; 5-methyl-4-octanol (96% pure). Cruentol was obtained from Dr. A. C. Oehlschlager (ChemTica, San Jose, Costa Rica) (release rate @ 25°C = 3 mg/d). Once collected, weevils were maintained in covered disposable polyethylene containers with sugarcane and separated by sex for no less than 18 h prior to each mating session. Adult insects for bioassay were sexed using dimorphic rostral and tibial char-

acteristics (Wattanapongsiri 1966). *Rhynchophorus cruentatus* is a relatively rare insect (Weissling et al. 1994a) and it was necessary to reuse the same weevils in repeated trials, except in the trials involving virgins. Insects were selected arbitrarily from containers for use. Weevils smaller than 29 mm were not used for male-female pairs and size assortive mating was not examined.

Mating was defined as the attempted insertion of the aedeagus of one male weevil while in contact with another female or male weevil. No assumptions were made about spermatophore transfer. Mating behaviors were observed by placing weevils in polystyrene arenas (Tri-State Plastics, Dixon, KY) (21 cm diam × 8 cm high) for 30 min sessions. Experiments were conducted under ambient fluorescent lighting (0.74 klux) at 22-26°C and 44-60% RH. In all experiments, two sexual stimulation indicators for males were measured: 1) the frequency of mating per 30 min session and 2) the time from placement into the arena until mating was initiated.

Experiment 1. Field-collected Weevils: Description of the Mating Sequence of Sequestered Pairs; Observation of Behaviors in Simulated Aggregations and Comparison of Sexual Stimulation Factors for Sequestered Versus Artificially Aggregated Males

A. Ethogram. Preliminary examination of weevil pairs allowed for the preparation of an ethogram. This preparatory ethogram was quantified using observations of the complete mating sequence of the 20 sequestered males in experiment 1C.

B. Observation of behavior in aggregations. Initially, 10 trials of scan sampling were conducted and some general observations were made about mating tendencies in aggregations. An ethogram of behaviors in the group environment was not prepared because the pace of activity in these arenas was so frenetic that quantification of any single behavior was impossible.

C. Comparison of the two sexual stimulation indicators for sequestered versus artificially aggregated males. Comparisons were made for the frequency of mating and the time to begin mating between 20 sequestered males and 20 focal males in an artificial mating aggregation. The artificial aggregation was assembled by simultaneously placing one randomly selected male weevil that was marked with a small amount of metallic marker (Sanford silver coat, Bellwood, IL) on the pronotum, with nine other males and 10 females into an arena. Male sexual stimulation indicators were then compared between sequestered and artificially aggregated males as previously described.

Experiment 2. Virgin Weevils: Comparison of Sexual Stimulation Indicators for Sequestered Versus Artificially Aggregated Males

Experiment 1C was repeated with virgin weevils. Twenty replications with sequestered virgin male-female pairs were compared to marked focal virgin males in artificial aggregations, 15 replicates.

In order to isolate the factors responsible for male sexual stimulation in groups, experiments 3, 4, 5 and 6 were conducted using the same containers in a different arrangement. A mating arena consisted of two containers stacked one on top of the other, open sides together, with a barrier separating them (Fig. 1). The lower chamber contained the treatment and the mating pair under observation was placed in the upper chamber, moving about on the barrier. This created a fractional aggregation. Barrier components and upper chambers were washed with soap and water and dried after each repetition. Arena placement was randomized for each replicate of the following experiments.

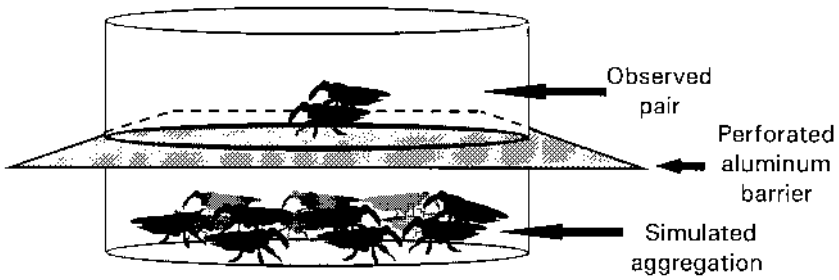

Fig. 1. Schematic diagram of the fractionally aggregated mating arena used in experiments 3 and 4. In experiment 5 only, glass barriers replaced the perforated aluminum. In experiment 6, synthetic aggregation pheromone replaced the weevils in the lower chamber.

Experiment 3. Comparison of Sexual Stimulation Indicators for Sequestered Versus Fractionally Aggregated Males

This experiment involved two treatments and tested whether semiochemical, tactile or visual cues influenced the frequency and celerity of mating for upper-chamber males. A barrier assembly consisted of a sheet of pierced aluminum (1.5 mm thick, 2 mm diam perforations, 12 holes/cm^2) overlaid with black polyester open-weave stretch fabric. The design of this arena allowed for the diffusion of volatiles into the observation chamber, while preventing the mating pair from seeing or touching weevils below. Chambers were allowed to equilibrate for 30 min immediately prior to each trial. The fractionally aggregated arena (treatment 1) held nine females and nine males in the lower chamber, with the mating pair under observation in the upper chamber, simulating the original group of 20 total weevils in experiment 1C. The lower chamber of the control arena (treatment 2) was left unoccupied. There were 20 replications of each treatment.

Experiment 4. Comparison of Sexual Stimulation Indicators for Fractionally Aggregated Males by Sex of Semiochemical Source

Three fractional aggregation arenas were arranged as described for experiment 3 to test whether semiochemicals from males, females or both were important in stimulating the mating behavior of the male in the chamber above. The treatments were: nine males plus nine females, 18 females, and 18 males. There were 20 replications of each of the three treatments.

Experiment 5. Comparison of Sexual Stimulation Indicators for Fractionally Aggregated Males by Sight and Sound of Other Weevils

To test whether the sight of or vibrations from other weevils were important stimuli, sheets of glass (3.5 mm thick) were used as barriers in place of the fabric and aluminum used in experiments 3 and 4. The glass prevented volatiles from the treatment chamber below from entering the upper chamber. Treatment 1 consisted of a clear glass barrier with nine male plus nine female weevils below creating a situation

where the pair could see and feel vibrations from the lower group but not sense semiochemicals produced by the group. Treatment 2 utilized clear glass with a vacant lower chamber exposing the weevil pair to no group-produced stimuli. Treatment 3 used the 18-weevil arrangement of treatment 1, but with painted glass as the barrier, allowing only vibrational cues to be received. This glass sheet was sprayed with matte black enamel (Rust-oleum Corp., Vernon Hills, IL) on the side facing the lower chamber 3 wks prior to use to allow the surface to fully dry and ventilate.

Experiment 6. Comparison of Sexual Stimulation Indicators for Pheromone in Fractionally Aggregated Chambers

The effects of synthetic aggregation pheromone on mating behavior of weevils were examined. There were three treatments: two doses of (±) cruentol and a control (no cruentol). The aluminum-fabric barriers were used in each mating arena. One-µl capillary glass tubes (Drummond Scientific Co., Broomall, PA) were filled with cruentol and then secured upright in Seal-ease tube sealer and holder (Becton Dickenson & Co., Rutherford, NJ). An equal amount of the Seal-ease was placed in the lower chamber of the control arena. The cruentol-containing arenas held either one or five microcaps (release rate = 3.02 ± 0.92 SD ng/h/µcap). The release rate was calculated by placing ten 1 µl microcapillary tubes in Seal-ease in the lower chamber of an arena with an aluminum-fabric barrier and upper chamber cover. After 24 h, the amount of cruentol lost from each tube was measured to determine the mean release rate. Cruentol was handled with disposable gloves and stored in a -10°C freezer when not in use. Weevils and cruentol were introduced into the testing room in separate, tightly closed containers. Arenas were randomized and placed 2 m apart. Cruentol was introduced into the lower chambers of arenas and was allowed to equilibrate for 3 min immediately prior to introduction of weevils into the upper chamber. After each replicate, lower chambers were quickly covered with lids to minimize diffusion of cruentol into the room. There were 20 replicates of each experiment.

Statistics

A preliminary scatterplot of the time to begin mating in experiment 1 suggested that mating in the first 250 sec was optimal for measuring sexual stimulation between sequestered and aggregated males. Therefore, the number of males mating within the first 250 sec was used for analysis by the Kruskal-Wallis (chi-square approximation) test (SAS Institute 1985). The number of apparent matings per 30 min observation period were square root transformed ($x + 0.5$) and analyzed by analysis of variance using PROC ANOVA (SAS Institute 1985). Least significant difference tests (SAS Institute 1985) were used for means separation where significant differences occurred.

Results and Discussion

Experiment 1. Field-collected Weevils: Description of the Mating Sequence for Sequestered Pairs; Observation of Behaviors in Artificial Aggregations and Comparison of Sexual Stimulation Factors for Sequestered Versus Artificially Aggregated Males

A. Ethogram. Of the 20 sequestered pairs studied for sequence of mating events, 14 mated as previously defined. All males and females made numerous physical contacts with one another whether or not they later mated. A summary of the sequence

of mating events is presented in the ethogram in Fig. 2. All males engaged in rostral rubbing, defined as touching the female's elytra with the distal tip of the rostrum and moving it in a serpentine pattern from the pygidium forward. A similar behavior has been observed in *Ips* beetles by Birch (1978) who suggested that this is a placating gesture. Another possible explanation for this behavior is that the weevils are discriminating heterospecific cuticular hydrocarbons (Takahasi & Gassa 1995). Five of the 20 male weevils did not engage in any behaviors other than the general physical contact and rostral rubbing (Fig. 2). The sixth male that did not mate moved directly from rostral rubbing to guarding, described below. Of the males that did mate, all followed a stereotyped sequence of behaviors. Both during and subsequent to the rostral rubbing the male tapped his antennae on the elytra and pronotum of the female as he began to mount her (Fig. 2). This antennal tapping and the actual mounting of the female was the first indication that a copulatory event was about to take place. There were not any other discernible behaviors that could be identified as "courtship". The antennal tapping was immediately followed by attempts to insert the aedeagus (Fig. 2). In all cases, the female remained very still during mating (Fig. 2). After a period of time (approximately 2 min) she initiated the termination of mating by moving her legs and walking across the arena floor. All of the males that mated maintained close physical proximity to the female, referred to here as guarding (Fig. 2). In most cases, the male was able to grasp the female with all six of his legs, remaining on top and riding on her back as she moved about the arena. If he grasped with only two or four of his legs he was dragged around with his pygidium scraping the arena floor. The guarding behavior might serve to deter mating attempts by other males in an aggregation, to stimulate the female to oviposit more quickly or to reduce the likelihood that the female will accept another partner (Eberhard 1996). Ten of the 14 males that mated proceeded from guarding the female to a second copulatory event (Fig. 2). There was not always the same level of female cooperation in subsequent attempts. Of the 10 males which went on to attempt a second mating, six attempted a third time and two of these attempted a fourth time. The sequence of virgin mating behaviors observed in experiment 2 was as illustrated in the ethogram (Fig. 2), except that some of the virgins did not guard their female partners or they separated immediately after mating, returning after several seconds to guard her.

B. Observation of behavior in aggregations. Male weevils seemed to be stimulated to mate while in a mixed-gender group. This phenomenon was originally noticed when weevils were being transported from collection sites, before they had been separated according to sex. These original observations led us to hypothesize that males were more highly stimulated to mate in aggregations.

In artificial aggregations, weevils engaged in frequent and apparently deliberate physical contact irrespective of gender. Males scrambled to obtain females quickly and there was a frenetic quality to the activities. Both males and females multiply

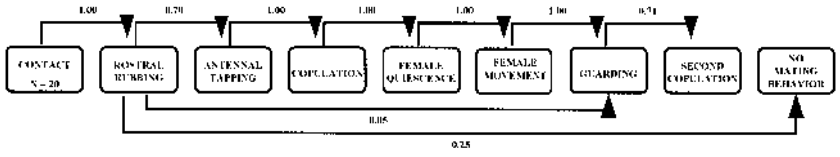

Fig. 2. Ethogram of *Rhynchophorus cruentatus* mating behavior from experiment 1A. Values represent percentage of mating pairs that proceeded to the next activity, as indicated by arrows.

mated. Males attempted to mate with other males in scan sampling and 13.9% of all mating attempts (n = 84) were homosexual in experiment 1C. Clusters of two to five males were observed attempting to mate with one female while other females were ignored. Males readily diverted their attentions from one female to others in the vicinity. Rival males attempted to supplant mating males (often successfully) by inserting their rostrums between the bodies of the male and female. The challenge was occasionally followed by venter to venter "wrestling" between males, but most males simply moved on in search of other females.

C. Comparison of the two sexual stimulation indicators between sequestered and artificially aggregated male weevils. Artificially aggregated *R. cruentatus* males were more highly stimulated to mate than males in sequestered pairs. The frequency of mating (P = 0.0001) and the number of males that mated in 250 secs or less (P = 0.0049) (Table 1) were significantly greater for the aggregated males. The heightened sexual stimulation of male weevils in groups is probably an adaptation to the increased competition for females in mating aggregations.

Experiment 2. Virgin Weevils: Comparison of Sexual Stimulation Indicators for Sequestered Versus Artificially Aggregated Males

The frequency of mating was significantly greater for artificially aggregated virgin males than for sequestered virgins (P = 0.001). The number of males that mated in the first 250 secs was also significantly greater for the aggregated virgins (P = 0.0132) (Table 1).

Experiment 3. Comparison of Sexual Stimulation Indicators for Sequestered Versus Fractionally Aggregated Males

In this experiment, the ability to see and to physically interact with the 18 other weevils below him was removed from the focal male's experience, although he was still able to receive olfactory and potential acoustical feedback from them. The mean frequency of mating was significantly higher for the males above the occupied chamber than for males above the vacant chamber (P = 0.0001) (Table 1). This suggested that vibrational and/or semiochemical stimuli were inducing the increased sexual activity. There was no difference between the sequestered and the fractionally aggregated males for the number of males which mated within 250 secs (P = 0.0803).

Experiment 4. Comparison of Sexual Stimulation Indicators for Fractionally Aggregated Males by Sex of Semiochemical Source

Males did not mate more often when placed above only males, only females or a combination of both (P = 0.1567). There was no significant difference in the number of males which mated in less than 250 secs (P = 0.0806) (Table 1).

Experiment 5. Comparison of Sexual Stimulation Indicators for Fractionally Aggregated Males by Sight and Sound of Other Weevils

There were no differences in the number of mating events or the time to begin mating for any of the treatments using glass as a barrier to prevent semiochemical stimulation of males. No males began mating in less than 250 secs, although the weevils appeared to experience some difficulty gaining traction on the glass barrier surfaces that might have contributed to their slower starts. Overall, the frequency of mating

TABLE 1. MATING RESPONSE OF *RHYNCHOPHORUS CRUENTATUS* MALES TO ARTIFICIAL AGGREGATIONS AND TO TACTILE, VISUAL AND SEMIOCHEMICAL CUES.

Experiment	Barrier Type	Treatment Chamber	No. Males Mating[1] in ≤250 sec (± SE)	No. of Matings[2] per 30 min (± SE)
1C	No barrier	Field-collected		
		Artificially aggregated	0.50 ± 0.1*	4.50 ± 0.7a
		Sequestered	0.10 ± 0.1	1.75 ± 0.3b
2	No barrier	Virgin		
		Artificially aggregated	0.30 ± 0.1*	4.87 ± 0.9a
		Sequestered	0	0.65 ± 0.2b
3	Perforated aluminum	9 males + 9 females	0.25 ± 0.1	4.10 ± 0.7a
		Vacant	0.05 ± 0.1	0.95 ± 0.2b
4	Perforated aluminum	18 males	0.25 ± 0.1	5.30 ± 0.6a
		9 males + 9 females	0.60 ± 0.1	4.70 ± 0.6a
		18 females	0.40 ± 0.1	3.60 ± 0.4a
5	Unpainted glass	9 males + 9 females	0	1.15 ± 0.4a
	Unpainted glass	Vacant	0	0.95 ± 0.4a
	Painted glass	9 males + 9 females	0	0.75 ± 0.2a
6	Perforated aluminum	Cruentol: 30.20 ng/h[3]	0.35 ± 0.1*	2.95 ± 0.7a
		Cruentol: 6.04 ng/h	0.05 ± 0.05	1.70 ± 0.4ab
		Vacant	0.05 ± 0.1	1.15 ± 0.4b

[1]Probability of obtaining chi square (Kruskal-Wallis test, chi square approximation) <0.05, as indicated by asterisk.

[2]Data transformed by $(x+0.5)^{0.5}$ to approximate homogeneity but are presented nontransformed. For each experiment, means within a column followed by the same letter are not significantly different according to ANOVA (P>0.05; LSD).

[3]± cruentol release rates were estimated as described in materials and methods.

per session was consistent with that of the weevils in arenas with vacant treatment chambers in experiments 3 and 6, suggesting that volatiles emanating from below may influence mating behaviors. The lack of difference in response among weevils in this experiment indicates that sexual stimulation of male weevils is not substantially influenced by sight alone of other mating weevils (although males often approach mating pairs in an artificial aggregation).

Experiment 6. Comparison of Sexual Stimulation Indicators for Fractionally Aggregated Males by Exposure to Synthetic Aggregation Pheromone

The frequency of mating response was significantly greater for the higher-dose group than for the group exposed to lower levels or to no cruentol ($P = 0.0438$). More males mated in a shorter time period when exposed to the higher level of cruentol than when exposed to the lower cruentol level or to no cruentol ($P = 0.0077$) (Table 1). The aggregation pheromone cruentol may serve a secondary function for *R. cruentatus* males: its presence appeared to stimulate them to mate more quickly and more frequently. This would most likely be an evolutionary adaptation to sexual selection pressures posed by the aggregation mating system. A male is cued by cruentol to the presence of other males and he increases his level of sexual activity in order to remain competitive for access to females. Further study is necessary to establish whether other semiochemicals are important in affecting the mating behavior of males of *R. cruentatus* in aggregations.

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ESTABLISHMENT OF *AGENIASPIS CITRICOLA* (HYMENOPTERA: ENCYRTIDAE) FOR BIOLOGICAL CONTROL OF *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE) IN FLORIDA

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ABSTRACT

The parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae), originally from Thailand and obtained from Australia, was released at 52 sites in Southwest Florida between May 1994 and September 1995 as part of a statewide program of biological control of citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Establishment and over-wintering was confirmed during spring of 1995 and 1996 in spite of frosts experienced during the previous winters. Parasitism from *A. citricola* at monitored groves increased from 2% in May 1994 to 86% in October 1995, apparently unhindered by native parasitoids. In contrast, apparent parasitism of citrus leafminer from endemic parasitoids fell from 30% to 2% during the same period. Wind-aided dispersal of *A. citricola* occurred in all directions and was documented to a maximum of 48 km from the nearest release point. *A. citricola* is now ubiquitous throughout the region and will probably remain a permanent component of the entomophagous complex using citrus leafminer.

Key Words: citrus leafminer, *Ageniaspis citricola*, biological control, *Phyllocnistis citrella*

RESUMEN

El parasitoide *Ageniaspis citricola* (Hymenoptera: Encyrtidae), que originalmente es de Tailandia pero qué fue obtenido para este proyecto en Australia, fue liberado en 52 sitios de la zona citrícola del sudoeste de Florida entre Mayo de 1994 y Septiembre de 1995 como parte de un programa estatal del control biológico del minador de los cítricos, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Durante las primaveras de 1995 y 1996 se confirmó su establecimiento a pesar de algunas heladas durante los inviernos anteriores. Parasitismo por *A. citricola* en plantaciones monitoreadas aumentó del 2% en Mayo de 1994 al 86% en Octubre de 1995, aparentemente sin impedimento por parasitoides nativos. En cambio, parasitismo del minador por parasitoides nativos bajó del 30% al 2% durante el mismo período. Se documentó la dispersión de *A. citricola*, aparentemente por medio del viento, en todas direcciones, con el máximo de 48 km de distancia del sitio de liberación más cercano. Ya se encuentra *A. citricola* en toda la región y se espera que sea un componente permanente del complejo entomófago que utiliza a *P. citrella*.

Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was detected in May, 1993 infesting Persian limes in Dade County, Florida (Heppner 1993). The moth spread quickly across Florida, and within several months was found in all citrus growing regions of the State (Knapp et al. 1993). It has since been de-

tected throughout the Caribbean, Central America (Heppner 1995), South America, and most Mediterranean countries (Knapp et al. 1993, Hoy and Nguyen 1997). Citrus leafminer had previously been reported as a major pest of new flush in southeast Asia, Australia, Japan, Taiwan, and South Africa (Heppner 1995). Larvae feed by mining the epidermal layer of leaves (Sonhi and Verma 1965), stems, and occasionally fruit.

Evidence from the United States, India, Japan, and Israel indicate that citrus leafminer can be managed by biological control from native and introduced parasitoids (Peña et al. 1996, Batra and Sandhu 1981, Ishii 1953, Argov and Rossler 1996). Surveys in 1993 and 1994 revealed that the leafminer was attacked by a complex of native parasitoids in the families Eulophidae, Encyrtidae, Elasmidae, Eurytomidae and Pteromalidae (LaSalle and Schauff 1996). Peña et al. (1996) found that generalist native parasitoids provided low to moderate levels of *P. citrella* parasitism in lime orchards in southeast Florida. They suggest that this reduction in effectiveness of native parasitoids may be the result of pesticide applications for the control of citrus pests. Given the apparent low levels of control by native parasitoids and difficulty in achieving good chemical control due to the biology of citrus leafminer (Knapp et al. 1995) the importation and release of additional biological control agents was deemed necessary.

Citrus leafminer had been detected in Australia in 1912 (Beattie 1993), and the gregarious parasitoid *Ageniaspis citricola* Logvinovskaya from Southeast Asia was introduced there in 1989 (Neale et al. 1995). *Ageniaspis* was imported from Australia to Florida with permission from that government in spring 1994. Federal and Florida state permits to release *A. citricola* from quarantine were granted for adults field-collected as pupae in May of that year (Hoy & Nguyen 1994). *A. citricola* is an internal koinobiont parasitoid of Citrus leafminer. Eggs are laid into eggs or first instar leafminer and parasitoids pupate inside the host prepupa, located within its pupal cell (Pomerinke, personal observation). The only reported host of *A. citricola* is Citrus leafminer (Hoy 1994), although development in the Mahogany leafminer, *Phyllocnistis erechtiisella* Chambers, has been observed (Stansly, unpublished data).

The following study was conducted to document establishment and dispersal of A. citricola, and its effects on incidence of native parasitoids. We report results of 13 releases at 10 groves (a total of 2,625 *A. citricola* adults) in 1994, and 39 releases at 25 groves (a total of 9,757 *A. citricola* adults) in 1995 made in five counties in Southwest Florida: Henry, Lee, Collier, Charlotte, and Glades.

Materials and Methods

Parasitoids were received from Gainesville, Florida, rearing facilities in plastic 50 ml vials containing an average of 100 adults. Wasps were provided with strips of filter paper moistened with pure honey and packed in Styrofoam boxes ($21 \times 21 \times 31$ cm) cooled with EverCold® gel refrigerant or Freez Pak Icicle®. Shipments were made by overnight courier and arrived midmorning the following day.

Release sites for *A. citricola* where chosen partly on the basis of a questionnaire which queried growers in the five county area on location, block size, variety, leafminer status, management practices and their willingness to suspend all pesticide applications for one year post-release. In 1994, 1995 and 1996, 13, 39 and 2 releases were made in 10, 25 and 2 groves respectively. Releases were generally made an hour or so before dusk, in trees with ample emerging foliage on which eggs and young Citrus leafminer were present. Vials were opened and wasps allowed to crawl out onto young leaves introduced into the opened neck of the vial to facilitate transfer in the field.

Sampling for evidence of leafminer pupae parasitized by *A. citricola* was conducted at least once, two weeks after each release in 1994 to confirm a first generation. No at-

tempt was made to confirm establishment at 1995 and 1996 releases sites because close proximity to previous release sites would have made it difficult to distinguish between establishment and immigration.

Establishment and Apparent Parasitism

Four 1994 release sites near Immokalee were monitored bi-weekly in 1994 and monthly in 1995 and 1996: Youngquest grove in Lee County, Foundation and Corkscrew groves in Collier County and A. Duda & Sons grove in Hendry County. An additional location, Rosbough grove, directly west of Corkscrew grove was added in late 1994 after *A. citricola* was found to have dispersed there. All groves were sampled for confirmation of establishment and overwintering.

Sampling of *A. citricola* was conducted from time of release/discovery at monitored release blocks through late October when citrus normally becomes dormant (Jackson 1991), and reinitiated in May 1995 and 1996. Samples were obtained by examining 100 intact pupal chambers of citrus leafminer chosen at random or the number encountered in 30 minutes. Pupal chambers were sampled since *A. citricola* and native parasitoids pupate outside the host co-incident with or previous to host pupation. The "sausage link" pupae of *A. citricola* within the host pre-pupal skin of the last host larval instar were easily distinguished from pupae of leafminers or native parasitoids, all of which were noted (Fig. 1). The resulting estimate of parasitoid incidence provided a convenient measure for comparing parasitoid activity among groves (Southwood 1978).

Dispersal

Dispersal was documented by surveying 23 citrus groves for the presence of *A. citricola* outward along 2 transects originating from each of 6 release sites between July and October, 1995. Intact citrus leafminer pupal chambers were examined at all encountered groves for 30 minutes or until *A. citricola* was discovered, whichever came first. Transects were followed until the end of contiguous grove was reached or another release site was encountered and ranged from 1.6-30 miles (2.6-48.3 Km).

Grove structure

Release sites were categorized as either single or multiple aged groves. Multiple aged groves contained blocks of trees consisting of the original planting, usually 15 years or older, along with resets of various ages comprising approximately 50% of the trees. A grove with 60% or more of the trees being uniform in size and age was considered single aged. By these criteria, 6 of the 11 release sites in 1994 were multiple-aged and 5 were single-aged blocks. In 1995, 4 of 17 release sites were multiple-aged and 13 single-aged.

Weather

Weather stations were monitored at two sites; Corkscrew grove and at the University of Florida's Southwest Florida Research and Education Center adjacent to the Foundation grove near Immokalee, Florida. Temperature and humidity (HMP35C probe), wind direction (024A probe) and speed (014B probe) were continuously recorded using a CR10 weather station (Campbell Scientific, Inc. Logan Utah 84321) at both sites in 1995 and 1996.

Fig. 1. Pupae *Pnigalio minio* (Walker) (top), *Ageniaspis citricola* Logvinovskaya (middle), and *Phyllocnistis citrella* Stainton (bottom).

RESULTS

Weather

Climatic conditions for 1995 and 1996 at the research center and 1996 at Corkscrew grove are presented in Table 1. Temperatures at the research center ranged from highs of 36.1°C and 35.5°C in August 1995 and July 1996, to lows of -1.6°C and -2.7°C in February 1995 and 1996 respectfully. Highs at Corkscrew grove reached 36.2°C in September, and dropped to -2.5°C in February. On February 4th, 17th, and 18th, 1996, three freezes were recorded with temperatures at -2.4, -1.9, and -0.9°C respectively at Corkscrew grove, and -2.7, -2.2, and 0°C respectively at the research cen-

TABLE 1. MAXIMUM, MINIMUM AND AVERAGE TEMPERATURE (C°) ALONG WITH AVERAGE RELATIVE HUMIDITY (%) AT SOUTHWEST FLORIDA RESEARCH AND EDUCATION CENTER, IMMOKALEE, FLORIDA IN 1995 AND 1996, AND CORKSCREW GROVE, COLLIER COUNTY, IN 1996.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Southwest Florida Research and Education Center—1995												
Maximum	27.7	30.0	31.1	32.2	35.0	35.0	35.0	36.1	34.4	33.3	31.6	28.8
Minimum	1.6	-1.6	0.0	10.5	17.2	16.6	21.1	21.1	21.6	15.5	5.0	-0.5
Average	16.6	17.2	20.3	23.0	26.6	26.6	27.5	28.0	27.7	25.8	19.7	16.9
Avg RH	80.0	86.2	85.8	87.0	84.4	82.9	82.5	83.2	84.7	87.0	78.7	NA
Southwest Florida Research and Education Center—1996												
Maximum	28.8	30.0	30.5	33.8	32.7	34.4	35.5	34.4	35.0	32.2	31.1	29.4
Minimum	-1.1	-2.7	1.1	7.2	16.6	16.1	20.5	19.4	18.8	11.1	5.5	3.8
Average	16.6	16.4	18.0	21.6	25.5	26.3	28.3	27.7	27.2	24.1	20.7	18.3
Avg RH	80.8	75.7	73.7	74.1	85.0	83.0	80.0	84.2	83.0	84.1	77.6	80.0
Corkscrew—1996												
Maximum	30.7	31.3	35.0	34.0	35.3	34.6	35.8	35.6	36.2	32.7	32.0	30.7
Minimum	10.6	-2.5	1.1	6.2	15.6	14.7	19.0	18.9	18.0	10.5	10.1	2.3
Average	18.8	16.9	17.8	21.3	24.8	25.6	27.3	26.1	26.2	23.4	19.8	17.5
Avg RH	84.5	79.5	77.8	79.7	84.0	88.0	86.4	89.4	86.7	89.0	83.9	84.6

NA, not available.

ter. An additional freeze was recorded at the research center on January 9th dropping temperatures to -1.1°C. No freeze lasted more then 9 hours.

Average relative humidity at the research center ranged from 87% in April to 73.1% in November in 1995 and 85% to 79.9% in April and March respectively in 1996 (Table 1). Average humidity at Corkscrew grove ranged from 79.5% in March to 89.7% in August, 1996.

Establishment

Wasps were often observed to immediately initiate searching movements accompanied by antennation, and to pause over leafminer eggs or small larvae, apparently to oviposit. Recoveries were made at five release sites in two counties during the May, 1995 survey (Table 2). Recoveries ranged from 10 of 25 pupal chambers yielding 29 *A. citricola* pupae to 2 of 25 yielding 6 *A. citricola* pupae. A mean 2.3 (SE = .13, N = 35) *A. citricola* pupae per pupal chamber were found in 1995.

During the May 1996 survey, A. citricola was recovered at 14 of 28 release sites in three counties (Table 2). Four recoveries were made at groves where *A. citricola* had been released in 1994 and recovered in 1995; the remaining 10 recoveries were made at 1995 release sites. Recoveries ranged from 41 of 100 pupal chambers yielding 94 *A. citricola* pupae to 1 of 100 yielding 2 *A. citricola* pupae. Again, a mean 2.3 (SE = .06, N = 145) A. citricola pupae were recovered from parasitized citrus leafminer pupae/ pupal chambers.

Apparent Parasitism

A detectable reproducing population of *A. citricola* was observed at Corkscrew grove 2 weeks after initial release in 1994. Incidence of *A. citricola* increased to 50% within 3 weeks and remained above 10% through 12 October, 1994, climbing to 83% by June 1995 (Fig. 2A). In contrast, parasitization by native parasitoids, in the genera *Pnigalio* and *Horismenus*, peaked at 30% 4 weeks following the release of *Ageniaspis*, and declined to 8% by June 1995.

Incidence of *A. citricola* at the Youngquest grove was documented on 30 May, 1995, at 10%, ultimately peaking at 90% by October 1995. In 1996, parasitism was documented at 20% on 20 May, peaked at 51% in June, falling to 41% by September. Incidence of *Pnigalio* and *Horismenus* fluctuated between 1% and 19% during the same time period, peaking in August, 1995 at 21% (Fig. 2B).

A. citricola was recovered on 12 October 1994, with an incidence of 1% in the Foundation grove, and again on 31 May 1995 at 2%, increasing to 51% by mid October. In 1996, *A. citricola* was recovered on 23 July with an incidence of 9%, and increasing to 50% by mid September. Parasitism by *Pnigalio* and *Horismenus* fluctuated between 3% and 8% during the same time period, with a maximum of 16% observed on August 9, 1995 (Fig. 2C).

A. citricola was first recovered at Duda grove on 28 November, 1994 with an incidence of 1%, increasing to 15% on 19 June, 1995 and peaking at 86% on 19 October, 1995. Parasitism from *Pnigalio* and *Horismenus* fluctuated between 1% and 40% during the same time period with maximum parasitism observed on 28 November 1994 at 40% (Fig. 2D).

A. citricola was discovered on 17 August 1994 at Rosbough grove, 3 months following their initial release at the adjacent Corkscrew grove. This was the first documented case of *A. citricola* dispersal from grove to grove in Southwest Florida. Incidence of *A. citricola* was 7% on August 17, peaking at 26% on October 6, 1994. In-

TABLE 2. RELEASE SITES, GROVE STRUCTURE, NUMBER OF *AGENIASPIS CITRICOLA* RELEASED, AND PARASITISM LEVELS FROM SAMPLES OF 25 AND 100 *PHYLLOCNISTIS CITRELLA* INTACT PUPAL CHAMBERS DURING MAY 1995 AND 1996 SURVEYS OF 1994 AND 1995 RELEASE BLOCKS, RESPECTIVELY.

Grove Structure[1]	Date	Total[2]	*Ageniaspis* 1995/1996[4]	CLM 1995/1996	Native Parasitoids[3] 1995/1996
MA	3 May 94	75	6/0	17/98	2/2
MA	16 May 94, 12 Apr 95	225	0/0	25/100	0/0
MA	10 Aug 94[5]	0	10/10	12/89	2/1
MA	26 Aug 94	200	9/2	11/95	5/3
MA	1, 25 May, 22 Jul 94	515	8/10	16/89	1/1
MA	20, 28 Sep 95	401	0	99	1
MA	19 Jul 95	300	2	82	16
MA	25 Oct 94	109	2/10	21/82	2/8
MA	28 Oct, 17 Nov 94; 22 May 95; 26 Jun 96	1289	0/0	25/100	0
MA	13 Sep 95	500	35	59	6
MA	7 Jul 95	600	4	80	16
SA	28 Feb 95	285	19	67	14
SA	23 Dec 94; 16 Mar, 20 Jul 95	750	0/0	20/95	5/5
SA	1, 17, 22 Mar, 31 May 95	1125	0	99	1
SA	26 Oct 94, 20 Jul 95	287	0/0	25/97	0/3
SA	25 Oct 94	119	0/0	18/100	7/0
SA	25 Apr, 9 Jun, 4 Aug 95	640	41	58	1
SA	17 May 95	100	3	95	2
SA	16 Jun 95	500	0	40	0
SA	21 Jun 95	235	0	40	0
SA	25 Oct 94	136	0/2	25/97	0/1
SA	16 May 95	300	9	87	4

1. MA = Multiple aged grove; SA = Single aged grove.
2. Total number of *Ageniaspis* released at each individual grove from 1994 to 1996.
3. Two most prevalent species Pnigalio and Horismenus.
4. Single numbers indicate that sampling was only conducted in 1996.
5. Date *Ageniaspis* was discovered at Rosbough grove in Lee county.

TABLE 2. (CONTINUED) RELEASE SITES, GROVE STRUCTURE, NUMBER OF *AGENIASPIS CITRICOLA* RELEASED, AND PARASITISM LEVELS FROM SAMPLES OF 25 AND 100 *PHYLLOCNISTIS CITRELLA* INTACT PUPAL CHAMBERS DURING MAY 1995 AND 1996 SURVEYS OF 1994 AND 1995 RELEASE BLOCKS, RESPECTIVELY.

Grove Structure[1]	Date	Total[2]	*Ageniaspis* 1995/1996[4]	CLM 1995/1996	Native Parasitoids[3] 1995/1996
SA	20 Jul, 4 Aug 95	600	1	89	10
SA	15 Aug 95	100	2	98	0
SA	20 Jan, 5, 22, 31 May 95	921	0	94	6
SA	1 Mar 95	150	1	98	1
SA	27 Jun 95	300	0	98	2
SA	28 Jun 95	300	0	99	1

1. MA = Multiple aged grove; SA = Single aged grove.
2. Total number of *Ageniaspis* released at each individual grove from 1994 to 1996.
3. Two most prevalent species Pnigalio and Horismenus.
4. Single numbers indicate that sampling was only conducted in 1996.
5. Date *Ageniaspis* was discovered at Rosbough grove in Lee county.

cidence of *A. citricola* increased over the 1995 season to a peak of 76% by October. High levels of parasitism were again recorded in 1996, reaching 85%. Parasitism from *Pnigalio* and *Horismenus* never exceed 7% and was almost undetectable in 1996 (Fig. 2E).

Grove structure

A. citricola was detected during the 1995 survey in 5 of the 7 multiple-aged release blocks but in none of the 4 single-aged release blocks (Table 2). The parasitoid was recovered at 7 of the 11 multiple-aged blocks and 8 of the 17 single-aged release blocks during the May 1996 survey (Table 2).

Nine groves where parasite establishment was not initially documented received more than one release between 1994 and 1996, 4 were multiple-age, the remaining 6 single-age. Establishment of *A. citricola* occurred in both types of grove structure with the probability of successful establishment being higher in multiple-aged groves (70%) then in single-aged groves (47%).

Dispersal

Dispersal of *A. citricola* was documented from July to October 1995. Maximum dispersal was documented on 25 October from Green Horizon grove in Collier County to Stoney's Citrus Groves, 16.1 miles (25.9 km) to the southwest (Fig. 3). In July, 1996 it was found through casual observation on Marco Island, 30 miles (48.2 km) south measured from Green Horizon Grove. *A. citricola* was recovered at all sample sites during the survey.

DISCUSSION

A. citricola appeared to establish more quickly in multiple aged blocks compared to single-aged blocks. Parasitoid survival may have been favored in multiple-aged

Fig. 2. Percent apparent parasitism of citrus leafminer pupae by *Ageniaspis citricola* and endemic parasitoids at five monitored groves. Samples taken weekly from 25 May to 21 Oct 1994, monthly from 1 May to 24 Oct 1995 and 23 May 1996 to Sep 1996.

blocks by the shelter and a clement microclimate provided by large trees, and a continual source of host material provided by rapidly growing small trees. In comparison, blocks of uniform age and size are characterized by synchronous flush, and a less stable microclimate that appeared to slow establishment of *A. citricola*. Number of releases required before establishment was documented also appeared to be an indicator of the importance of grove structure. Nevertheless, *A. citricola* eventually was established uniformly throughout the region, demonstrating that overall, conditions are favorable for this species.

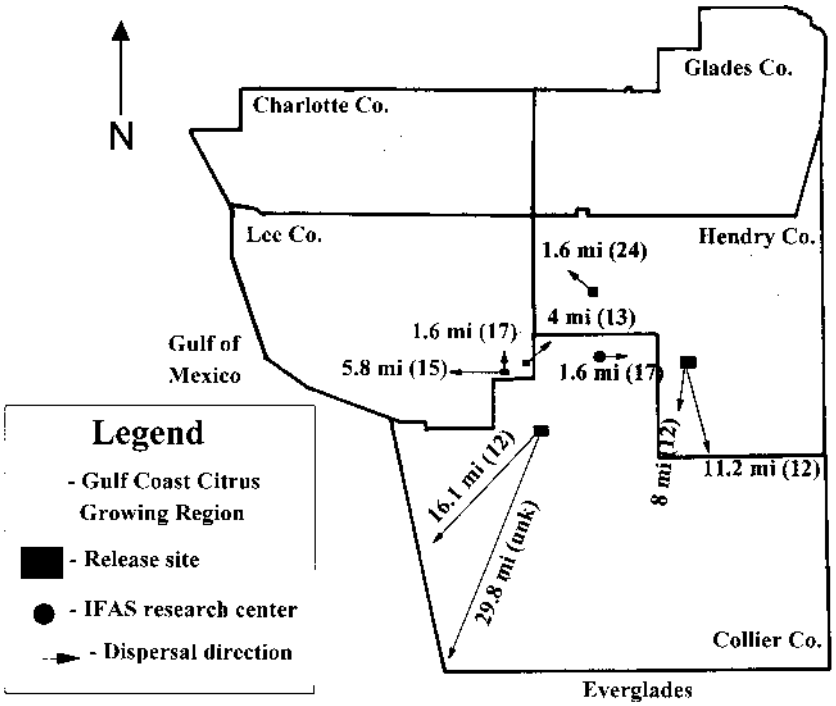

Fig. 3. Dispersal of *Ageniaspis citricola* documented from six release sites in 1996. Relative direction and distance depicted by arrows. Numbers in parenthesis indicate number of months recovery followed release. Arrows and map are not to scale.

These conditions include long growing season with hot, humid summers and mild winters despite two freezes in 1995 and four in 1996. Edwards and Hoy (in press) found that survivorship of *A. citricola* was best at humidities between 80% and 95%, within the average range for Southwest Florida during most of 1995 and 1996 (Table 1). Probability of establishment may have been enhanced by releasing early in the rainy season, May through August, maximizing the interval of warm, humid weather and ideal growing conditions.

Parasitism of citrus leafminer by *A. citricola* steadily increased at most sites following initial release, whereas the proportion of host pupal chambers with native parasitoids tended to decline (Fig. 2). *A. citricola* develops internally, pupates in the leafminer pupal chamber and probably does not have alternate hosts of ecological significance in the area, although there are at least 3 other species of *Phyllocnistis* present (unpublished data). In contrast, native parasitoids of citrus leafminer such as *Pnigalio* spp. etc. are generalists capable of parasitizing many different types of leafminers (Browning et al. 1996, Peña et al. 1996). Also, delayed internal development leaves hosts containing *A. citricola* open to parasitism from quickly developing external native parasitoids such as *Pnigalio* spp, unlikely to distinguish between parasitized and unparasitized citrus leafminer (Pomerinke, personal observation). Yet, in spite of these apparent disadvantages, *A. citricola* came to dominate the nitch provided by citrus leafminer.

Lack of alternate hosts means *A. citricola* populations must track host populations, themselves dependent on the cyclic patterns of flush, which is largely arrested during the winter (Jackson 1991) when generalist native parasitoids would have the greatest advantage. As a likely consequence *A. citricola* was largely absent during the May 1996 samples, whereas native parasitoids where relatively abundant. However *A. citricola* numbers later rebounded, apparently at the expense of native parasitoids. Possibly, the superior host-finding ability that comes with host specificity conferred a key advantage in the competition for hosts.

Superior searching ability was illustrated in the dispersal of *A. citricola* to new groves. In spite of impediments that a small size (approximately 1 mm in length according to Evans 1995 and Logvinoskaya 1983) should pose to directed flight, *A. citricola* was able to disperse over large distances, rapidly colonizing widely scattered citrus plantings over an extensive area (Fig. 3). *Ageniaspis* was found to have dispersed equivalent distances in the Indian river, Homestead, and Orlando citrus growing areas (Hoy et al. 1995). Dispersal distances in Southwest Florida are also consistent with documented dispersal in Louisiana (United States), Bahamas, Honduras, and Australia (Hoy and Nguyen 1997). In this ability *A. citricola* was not unlike its leafminer host which dispersed throughout Florida over a single growing season. Given successful reproduction, overwintering and dispersal throughout Southwest Florida, we feel confident that *A. citricola* has become a permanent addition to the fauna of the region, contributing a significant component of natural mortality to citrus leafminer populations.

Acknowledgments

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ALTERNATE HOST PLANTS OF COWPEA CURCULIO, (COLEOPTERA: CURCULIONIDAE) IN ALABAMA

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ABSTRACT

Cowpea curculio, *Chalcodermus aeneus* (Boheman), is an important pest of cowpea, *Vigna unguiculata* (L.) Walpers, in the southeastern United States. This insect also feeds on other fabaceous crops and a number of wild host plants. In a field survey done in Alabama during 1992 to 1994, adults of cowpea curculio were collected on 31 alternate host plant species representing 11 plant families, and eggs and larvae were collected on three fabaceous plant species of the subtribe Phaseolinae. Before the cowpea cropping season in the spring, some of the alternate host plants of adults included narrow-leaved vetch, *Vicia sativa* ssp. *nigra* (L.) Erhardt, purple cudweed, *Gnaphalium purpureum* L., heartwing sorrel, *Rumex hastatulus* L., cutleaf eveningprimrose, *Oenothera laciniata* L., and moss verbena, *Verbena tenuisecta* Briquet. In May and June, cowpea curculios reproduced on snapbean pods, *Phaseolus vulgaris* L., before cowpea plants bloomed, indicating that adults from this new generation could infest cowpeas during pod formation. Adults fed on sicklepod, *Senna obtusifolia* (L.) Irwin & Barneby, during the cowpea cropping season. After the end of the cowpea cropping season, cowpea curculio produced an overwintering generation on *Strophostyles umbellata* (L.) Elliott and *S. helvula* (Muhlenburg ex Willdenow) Britton. Adults overwintered in clumps of broomsedge, *Andropogon virginicus* L. Purple cudweed, heartwing sorrel, moss verbena, and sicklepod may represent new host records for cowpea curculio. Destruction of spring alternate hosts and overwintering hosts of cowpea curculio and crop rotation of cowpeas away from snapbeans may help to reduce cowpea curculio infestation in cowpea.

Key Words: insecta, cowpea curculio, *Chalcodermus aeneus*, alternate host plants

RESUMEN

El gorgojo del caupí, *Chalcodermus aeneus* (Boheman), es una plaga importante del caupí, *Vigna unguiculata* (L.) Walpers, en el sureste de los Estados Unidos. Este curculiónido también se alimenta de otros cultivos fabáceos y de un número de hospederos silvestres. Gorgojos adultos fueron obtenidos durante un muestreo de campo realizado en Alabama de 1992 a 1994 de 31 especies de plantas hospederas alternativas representando a 11 familias de plantas, y huevos y larvas del gorgojo fueron colectados en tres especies de plantas fabáceas de la subtribu Phaseolinae. Algunas de las plantas alternativas hospederas para los adultos del gorgojo utilizadas antes de la cosecha de la primavera del caupí incluyeron a *Vicia sativa* ssp. *nigra* (L.) Erhardt, *Gnaphalium purpureum* L., *Rumex hastatulus* L., *Oenothera laciniata* L., y a *Verbena tenuisecta* Briquet. En mayo y en junio, los gorgojos del caupí se reprodujeron en vainas de *Phaseolus vulgaris* L. antes de que las plantas de caupí florecieran, indicando que los adultos de esta nueva generación podrían infestar caupís durante la formación de la vaina. Se observó que gorgojos adultos se alimentaron de *Senna obtusifolia* (L.)

Irwin & Barneby, durante la temporada de la cosecha del caupí. Después de la temporada de la cosecha el curculiónido produjo una generación de invernación en *Strophostyles umbellata* (L.) Elliot y *S. helvula* (Muhlenburg ex Willdenow) Britton. Adultos invernaron en grupos de *Andropogon virginicus*. Es posible que *Gnaphalium purpureum*, *Rumex hastatulus*, *Verbena tenuisecta*, y *Senna obtusifolia* representen hospederos no reportados previamente para esta especie de curculiónido. Destrucción de hospederos alternos de la primavera y de los hospederos utilizados por este gorgojo para su invernación y rotación de cultivos del caupí lejos de *Phaseolus vulgaris* podría ayudar a reducir la infestación del gorgojo del caupí en cultivos del caupí.

Cowpea curculio, *Chalcodermus aeneus* Boheman (Coleoptera: Curculionidae), is one of the most economically important pests of cowpea, *Vigna unguiculata* (L.) Walpers (Fabaceae), in the southeastern United States. This insect caused more than $1.2 million in crop damage and control costs in Georgia in 1991 (Adams & Chalfant 1992). Cowpea is the preferred host plant of the cowpea curculio (Ainslie 1910, Arant 1938, Bissell 1938). Both larvae and adults of cowpea curculio feed on and damage cowpea plant tissue (Arant 1938).

Adults of both sexes damage ripening pods with their rostrum and females oviposit through pod walls into seeds. Larvae feed and develop in the seeds of ripening pods.

In the southeastern U.S., adult cowpea curculios have been collected from at least 21 species of plants from 10 plant families, while eggs and larvae have been collected from four species of the subtribe Phaseolinae (Fabaceae): snapbean, *Phaseolus vulgaris* L., deer pea, *Vigna luteola* L., *Strophostyles umbellata* (Muhlenburg ex Willdenow) Britton and *S. helvula* (L.) Elliott (A single larva was collected from cotton, *Gossypium hirsutum* L., in 1905, but Ainslee (1910) considered it an accidental occurrence) (Ainslee 1910, Arant 1938, Bissell 1938, Bissell 1939, Langston 1939, Bissell 1940, Hetrick 1947, Dupree & Beckham 1955). These alternate host plants may provide important nutritional resources and protective habitat to cowpea curculio before, during, or after the cowpea cropping season. However, little information is available about the seasonal incidence of cowpea curculio on these plants.

Alternate host plants play an important role in the biology of a number of crop pests (Headlee & McColloch 1913, van Emden 1981, Stadelbacher 1986, Fleischer & Gaylor 1987, Jones et al. 1992). Knowledge of the population dynamics of pests on alternate host plants can be used in the development of pest management strategies to reduce crop infestation.

Little is known about the seasonal incidence of cowpea curculio on wild host plants in Alabama. The objective of our three-year study was to identify the alternate host plant complex of cowpea curculio in Alabama, and to determine seasonal occurrence of cowpea curculio on these plants.

Materials and Methods

Potential alternate host plants of cowpea curculio were sampled from 1992 to 1994 at the Wiregrass Substation at Headland, AL, the E. V. Smith Research Center at Tallassee, AL, and at Auburn, AL, Gulf Shores, AL, and Fishing River Point, AL. A 0.4 ha field was planted with 'California Blackeye-5' cowpeas using standard agronomic practices, at both Headland and Tallassee in 1992 and 1993. Planting dates for cowpeas were 28 May at Tallassee and 1 June at Headland in 1992, and 30 April at Head-

land and 7 May at Tallassee in 1993. Naturally occurring weed species were allowed to grow unchecked in 20-m field borders around these cowpea fields. Plants in field borders were sampled biweekly, beginning in mid-April, and then weekly after cowpeas were planted. Weedy areas near additional cowpea fields were sampled at the Headland and Tallassee locations as well. Weekly sampling was continued through September in both 1992 and 1993 and through June in 1994. After the cowpea growing season, overwintering host plants were sampled on a monthly basis from November to March in 1992-93, and November to February in 1993-94.

Several species of wild legumes that did not occur at the two experiment stations were sampled. This sampling was done at Auburn (September and November 1993 and August and October 1994), at Gulf Shores (August 1994) and at Fish River Point (October 1992 and August 1994).

More than 127 species of plants from 29 plant families were sampled in this survey. Plant specimens were keyed using the keys of Radford et al. (1968) and Isely (1990). John Freeman, Department of Botany and Microbiology, Auburn University, AL, verified plant identifications.

Sweep net sampling and direct visual observations were used to sample for cowpea curculios on alternate host plants. Selection of sampling method was dependent on plant size, structure, and on the level of homogeneity of plant stands. In homogeneous stands of plants, a standard (38.1 cm diameter) insect net was used to sweep for adult cowpea curculios. The number of sweeps varied with the size of plant stands. When possible, 100 sweeps were taken per plant species. Direct visual observations were used when plant stands were (1) heterogeneous, (2) when physiognomy of plant stands would not permit effective sweep sampling or (3) when sweep sampling yielded adult cowpea curculios. Plants were examined visually for eggs, larvae, and adults of cowpea curculio. At least 10 plants per species were visually sampled when possible. The number of eggs, larvae and/or adults were recorded and specimens were transported to the lab for identification. The data presented herein represent the relative occurrence of cowpea curculio on alternate host plants in Alabama.

Results and Discussion

Of the 127 plant species sampled in the survey, 360 specimens of cowpea curculio (eggs, larvae and/or adults) were found on 31 species representing 11 plant families (Table 1). Adults were found on all 31 alternate host plant species either before, during, or after the cowpea cropping season. Eggs and larvae were found on only three fabaceous host plant species which were all members of the subtribe Phaseolinae (Iseley 1990). Average counts of cowpea curculio were difficult to compare statistically among plant species due to differences in sampling methods, phenology of host plants, physiognomy of host plant stands, homogeneity of host plant stands, and size of host plant populations.

Spring (before cowpea pod formation)

Alternate host plants provided food and/or shelter for cowpea curculio adults as they emerged from overwintering sites. In the spring, we collected adult cowpea curculios from 21 plant species or about two thirds of all of the plant hosts recorded in the survey (Table 1). More than half of all adults collected in this survey (total = 190), were found in the spring. Adults were observed resting at the base of plants and feeding on stems, flowers, pollen, pods, and extra-floral nectaries of the plants.

One of the first spring hosts on which adults were found was narrow-leaved vetch, *Vicia sativa* ssp. *nigra* (L.) Ehrhardt (Fig. 1). Narrow-leaved vetch was in vegetative

TABLE 1. PLANT SPECIES FROM WHICH *C. AENEUS* (CC) WAS COLLECTED IN ALABAMA, 1992-1994.

Family	Host Plant	Common Name	Dates of Collection	CC	Stage[1]	Site[2]
Poaceae	*Agropyron repens* (L.) Beauvois	quackgrass	29 Apr.-10 Sep.	5	A	H
(Gramineae)	*Andropogon virginicus* L.	broomsedge	23 Oct.-20 May	62	A	H, T
	Aristida stricta Michaux	wiregrass	25 Feb.-29 Apr.	7	A	H
	Digitaria sanguinalis (L.) Scopoli	large crabgrass	12 Aug.	10	A	T
	Paspalum urvillei Steudel	Vasey grass	7 Nov.-25 Mar.	3	A	T
Cyperaceae	*Cyperus esculentus* L.	yellow nutsedge	27 May, 10 Sep.	2	A	H
Polygonaceae	*Rumex hastatulus* Baldwin ex Elliott	sheep sorrel	27 Apr.-11 May.	25	A	H
Amaranthaceae	*Amaranthus retroflexus* L.	redroot pigweed	12 Aug	1	A	T
	Amaranthus spinosus L.	spiny amaranth	12 Aug	7	A	H, T
Fabaceae	*Cyamopsis tetragonoloba* (L.) Taub	guar	14 Sep.-24 Oct.	5	A	H
(Leguminosae)	*Desmodium tortuosum* (Swartz) D.C.	Florida beggarweed	2 Jun.-20 Aug.	8	A	H
	Lathyrus hirsutus L.	hairy pea	3 Jun.	5	A	T
	Phaseolus vulgaris L.	common bean	13 May-16 Jun.	46	E, L, A	H
	Senna obtusifolia (L.) Irwin & Barneby	sicklepod	18 Jun.-20 Aug.	12	A	H
	Strophostyles helvula (L.) Elliott	wild bean	20-23 Aug.	48	E, L, A	GS, F
	Strophostyles umbellata (Muhlenburg ex Willdenow) Britton	wild bean	28 Aug.-23 Oct.	30	E, L, A	AU
	Trifolium incarnatum L.	crimson clover	27 Apr-13 May	7	A	T
	Vicia sativa ssp. *nigra* (L.) Ehrhardt	narrowleaf vetch	22 Apr.-2 Jun.	32	A	H, T
	Vicia tetrasperma (L.) Schreber	wild lentil	22 Apr.-6 May	2	A	T

[1]E = eggs, L = larvae, A = adults.
[2]AU = Auburn, F = Fish River Point, GS = Gulf Shores, H = Headland, T = Tallassee.

TABLE 1. (CONTINUED) PLANT SPECIES FROM WHICH *C. AENEUS* (CC) WAS COLLECTED IN ALABAMA, 1992-1994.

Family	Host Plant	Common Name	Dates of Collection	CC	Stage[1]	Site[2]
Geraniaceae	*Geranium carolinianum* L.	carolina geranium	11 May	2	A	H
	Sida spinosa L.	prickly sida	12 Aug.	1	A	T
Onagraceae	*Oenothera laciniata* Hill	cutleaf eveningprimrose	5 May-2 Jun.	10	A	H, T
Convolvulaceae	*Ipomoea lacunosa* L.	pitted morningglory	16 Sep.	1	A	T
Verbenaceae	*Verbena tenuisecta* Briquet	moss verbena	20 May-2 Jun.	13	A	H
Asteraceae	*Acanthospermum hispidum* D.C.	bristly starbur	27 May-14 Aug.	5	A	H
(Compositae)	*Conyza canadensis* (L.) Cronquist	horseweed	12 Aug.	2	A	T
	Erigeron strigosus Muhlenburg ex Willdenow	daisy fleabane	13 May	2	A	H
	Eupatorium capillifolium (Lamarck) Small	dogfennel	25, 27 May	1	A	T
	Gnaphalium purpureum L.	purple cudweed	29 Apr.-27 May	22	A	H, T
	H*ypochoeris elata* L.	cat's ear	22 Apr.-11 May	5	A	H
	Taraxicum officinale Wiggers	dandelion	5 May	1	A	T

[1]E = eggs, L = larvae, A = adults.
[2]AU = Auburn, F = Fish River Point, GS = Gulf Shores, H = Headland, T = Tallassee.

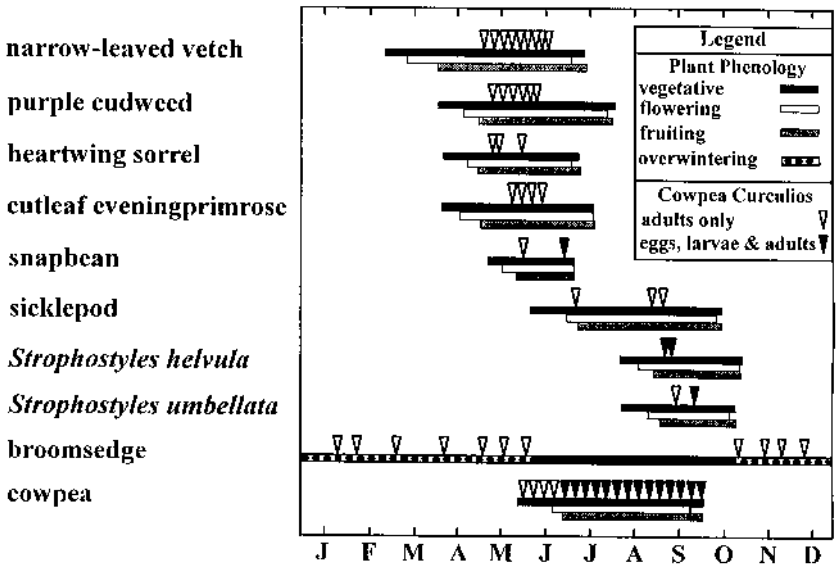

Fig. 1. Seasonal occurrence of *Chalcodermus aeneus* on selected host plant species and host plant phenology in Alabama, 1992-1994.

stage as early as late February and pod formation began as early as March. The earliest that adults were found on narrow-leaved vetch in this study was 22 April, the same date that Arant (1938) first found cowpea curculio adults on wild hosts at Auburn in the 1930's. Thirty-two adults were found on this plant from 22 April to 2 June, representing about 9% of the total adults collected. Adults occurred on narrow-leaved vetch before the formation of cowpea pods in mid-June (Fig. 1). The greatest number of adults per vetch plant was recorded in early May in 1993 and 1994 (Fig. 2). We found adults resting at the base of narrow-leaved vetch, feeding on pods, seeds, and nectar from extra-floral nectaries. Adults damaged pods and seeds, but no eggs or larvae were found in pods of narrow-leaved vetch during the survey. Some oviposition may have occurred in pods in the field, but it would be difficult for larvae to complete their cycle on narrow-leaved vetch due to rapid pod development and subsequent shattering of pods. The nectar and pods of narrow-leaved vetch may be a nutritional maintenance source for adults emerging from diapause in the spring before cowpea pod formation. Arant (1938) noted that it is important that adult curculios have access to alternate host plants in the spring because starvation is one of the leading mortality factors for adult curculios emerging from overwintering diapause. Narrow-leaved vetch plants often sprouted and grew among clumps of broomsedge, which provided overwintering sites for adults.

Adults were frequently collected from purple cudweed, *Gnaphalium purpureum,* in the spring (Fig. 1). Purple cudweed is one of the most commonly occurring spring weeds in southern Alabama (Jones 1961). This plant often grew near broomsedge and cowpea fields during this study, and we sampled it from April through June. Adults were found on purple cudweed at the base of the plant, on stems and feeding in the inflorescences on floral parts, pollen and/or nectar. A total of 22 adults were collected from purple cudweed from 29 April to 27 May, (Fig. 1), or about 6% of all curculios col-

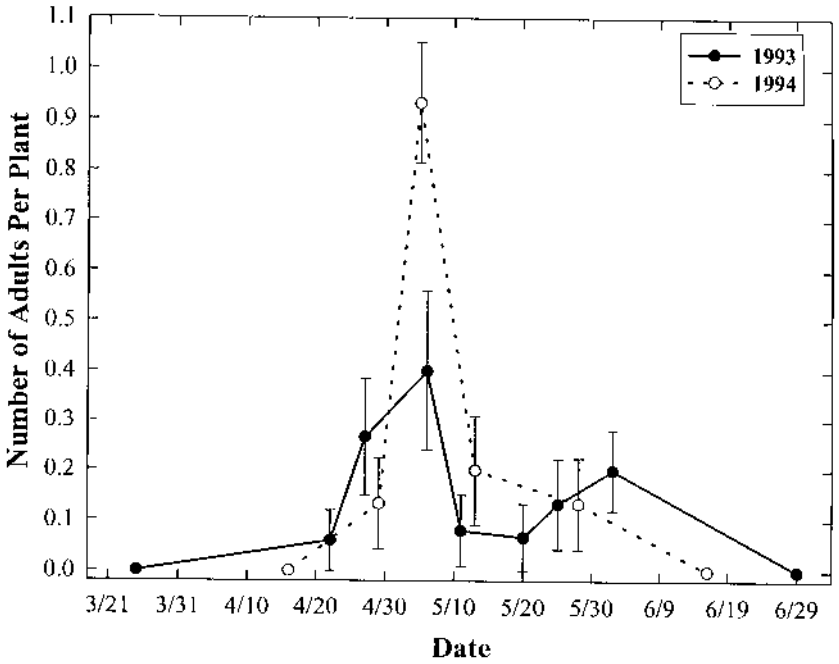

Fig. 2. Seasonal incidence of *Chalcodermus aeneus* on narrow-leaved vetch, *Vicia sativa* ssp. *nigra*, in Alabama, 1993-1994.

lected in the survey. The greatest number of adults per plant was 0.6 on 20 May in 1993 (Fig. 3). Cowpea curculio has not previously been recorded on purple cudweed. This plant may be another nutritional maintenance source for adults before the cowpea growing season.

In May and June, we also found adult curculios feeding on inflorescences of moss verbena, *Verbena tenuisecta* Briquet, and rough fleabane, *Erigeron strigosus* Muhlenburg ex Willdenow. These plant species were not previously documented as hosts for cowpea curculio.

Heartwing sorrel, *Rumex hastatulus* Baldwin ex Elliott is one of the most commonly occurring spring weeds in southern Alabama (Jones 1961). This annual plant species is closely related to the perennial sheep sorrel, *R. acetosella* L. Arant (1938) reported that adult curculios ". . . fed sparingly on sheep sorrel and evening primrose but the plants could hardly be considered a suitable source of food except in the early spring." Sheep sorrel did not occur in our study area, but we did sample heartwing sorrel around cowpea fields from late March to late June. We found a total of 25 adults on this species from 27 April to 11 May, representing about 7% of all curculios collected (Fig. 1). Most adults were found on the plant stems just below the soil surface.

Adult curculios were also collected from cutleaf evening-primrose in the spring from 5 May to 2 June. This plant was abundant around cowpea fields and is also a common spring weed in southern Alabama (Jones 1961). Adults were found at the base of the plant and feeding on the stems. Arant (1938) observed that adult curculios fed sparingly on this species in the early spring. We found a total of 10 adults on cut-

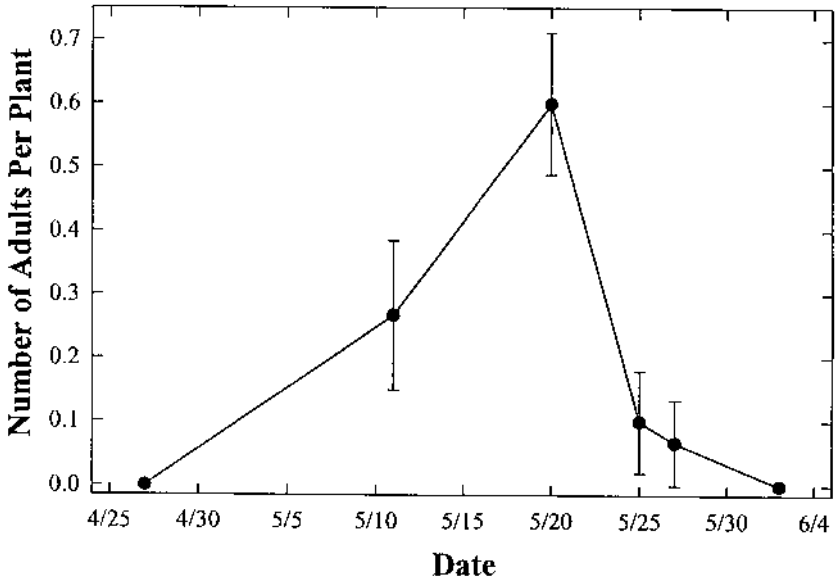

Fig. 3. Seasonal incidence of *Chalcodermus aeneus* on purple cudweed, *Gnaphalium purpureum*, in Alabama, 1993.

leaf evening-primrose from 5 May to 2 June, representing about 3% of all curculios collected (Fig. 1).

Snapbean, *Phaseolus vulgaris* L., was the only cultivated alternate host species from which we collected cowpea curculio. Snapbean is closely related to cowpea and belongs to the same family and subtribe. Cowpea curculio reproduces on snapbean and we found adults and the first eggs and larvae of the season on it. Pod formation had occurred on snapbean by 13 May when we collected 40 adults per 20 plants. On that date, adults were feeding on pods and hypocotyls and mating on the plants. Females oviposited on snapbean pods on or before 13 May which coincided with the earliest emergence of cowpea seedlings. Eggs and larvae were found in snapbean pods on 16 June when cowpea pods were beginning to form. These larvae eventually dropped out of the pods, pupated in the soil, and emerged as adults in July, when they infested cowpea fields. Other researchers have suggested rotating cowpea fields away from areas previously planted with snapbeans to avoid emerging adult curculios (Arant 1938, Hetrick 1947).

Summer (from cowpea pod formation to senescence)

Cowpea curculios were collected from 13 plant species during the period of cowpea pod formation and maturity (Table 1.). Only one adult cowpea curculio was found on each of eight of these species. In the summer, cowpea curculios were observed feeding on four alternate plant species: sicklepod, *Senna obtusifolia* (L.) Irwin & Barneby; *Strophostyles helvula* (L.) Elliott; *S. umbellatta* (Muhlenberg ex Willdenow) Britton; and guar, *Cyamopsis tetragonoloba* (L.) Taub. Few cowpea curculios were collected from alternate hosts during the period of peak cowpea pod formation from mid-June

to mid-August. Alternate host plants may have been less attractive to cowpea curculios during this period due to the presence cowpea pods.

Adults fed on sicklepod, one of the most commonly occurring summer weeds in southern Alabama (Jones 1961), in mid-June and mid-August (Fig. 1). Sicklepod occurred in and around cowpea fields and formed pods from early July to September. Adults were observed feeding on pods and stems in August, but no eggs or larvae were found in pods. Cowpea curculio has not previously been recorded from sicklepod.

Towards the end of the cowpea cropping season, cowpea curculio on *Strophostyles helvula* was found in late August at Gulf Shores and Fish River Point. Cowpea curculio was also found on *S. umbellata* at Auburn from late August to October. Eggs, larvae, and adults were collected on *S. helvula* and *S. umbellatta* wherever these plants occurred. About 21% of all cowpea curculios in this survey were collected from these two species (Table 1). Cowpea curculio successfully reproduces on *S. helvula* and *S. umbellatta* but the new generation of adults produced on this plant emerges after the cowpea harvest. However, *S. helvula* and *S. umbellatta* can act as a reproductive sink for cowpea curculios to produce the overwintering generation (Bissell 1938).

Cowpea curculio adults were found on guar, *Cyamopsis tetragonoloba* L., at Headland at the end of the cropping season. A total of five adults were found feeding on this plant (two on 14 September, and three on 24 October 1992). Adults fed on the pods of this plant which resemble cowpea pods. They were the only green pods available in the area after cowpeas had senesced. Cowpea curculio eggs or larvae were not found in guar pods. This species is not naturalized in the southeastern United States (Isely 1990) and cowpea curculio has not previously been recorded from this plant. Guar occurred only at the Wiregrass Substation as an adventive volunteer from previous variety trials and is probably not an important population sink for cowpea curculios in Autumn.

Overwintering

In the Autumn, adult curculios were found in cowpea leaf litter, and in clumps of vaseygrass, *Paspalum urvillei* Steudel, wiregrass, *Aristida stricta* L., and broomsedge, *Andropogon virginicus* L. (Table 1). Vasey grass and wiregrass have not previously been recorded as overwintering sites for cowpea curculio. Overwintering cowpea curculios were found on vaseygrass and wiregrass on only two dates for each species. Most of the overwintering adults in this survey were found in clumps of broomsedge; an observation also made in Georgia (Bissell 1940). Clumps of *Andropogon* spp. may provide protection from cold weather for several species of overwintering insects (Headlee & McCulloch 1913, Roach 1991). Sixty-two adults were collected from broomsedge at the Alabama study sites, representing 17% of all cowpea curculios collected in the survey. The mean number of adults per plant never exceeded 1.4 (Fig. 4). Overwintering adults were found in clumps of broomsedge from October to May but not during the cowpea cropping season when they are found in cowpea fields (Fig. 4). Adults were often found overwintering in the same clumps of broomsedge which had been overwintering sites in the previous year. It is unlikely that these were the same adults that overwintered the previous year because adults are not known to live for more than one year (Dupree and Beckham 1955). Several of the spring hosts such as narrow-leaved vetch and purple cudweed grew near clumps of broomsedge in the spring. Adults may move from these overwintering sites to adjacent spring nutritional hosts to renew the cycle.

Alternate host plants are important in the seasonal cycle of cowpea curculio and could be managed to reduce their abundance on cowpea. Our survey suggests that

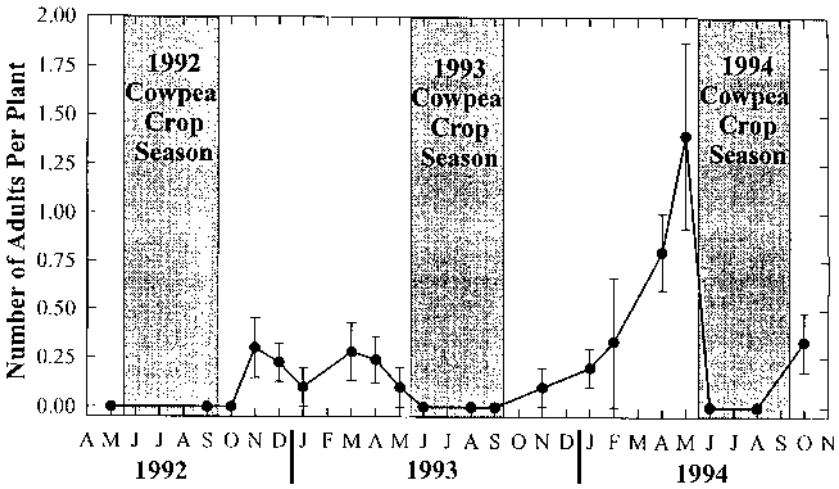

Fig. 4. Seasonal incidence of *Chalcodermus aeneus* on broomsedge, *Andropogon virginicus*, in Alabama, 1992-1994.

cowpea curculios use a variety of plants during their life cycle. Destruction of early-season hosts such as narrowleaf vetch and purple cudweed around cowpea fields might reduce the cowpea curculio's nutritional maintenance sources in the spring and break the link between overwintering diapause and the cowpea cropping season. Destruction of overwintering hosts such as broomsedge might reduce potential infestation sources and therefore reduce infestations in nearby cowpea fields. The rotation of cowpea fields away from snapbean fields might also reduce the infestation of cowpeas by the generation of cowpea curculios produced on early-season snapbeans. Future research could be directed towards testing optimal vegetational management strategies such as tillage, mowing, and herbicide application in alternate hosts and evaluating optimal crop rotation strategies.

ENDNOTE

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ASSESSMENT OF BIOLOGICAL CONTROL OF *BEMISIA TABACI* (HOMOPTERA: ALEYRODIDAE) ON COMMON BEAN IN HONDURAS

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ABSTRACT

Two experimental field trials assessed the effect of natural enemies on immature stages of *Bemisa tabaci* (Gennadius) infesting two varieties of common bean (*Phaseolus vulgaris* L.), 'Dorado 364' (Bean Golden Mosaic Virus (BGMV) tolerant) and 'Catrachita' (BGMV susceptible). Studies were carried out at Zamorano, Honduras during 'primera' (May-August) 1995 and 'postrera' (September-January), 1995-96. Treatments corresponded to two types of exclusion cages ($1 \times 1 \times 0.5$ m) and a no cage treatment. Cages were covered with organdy to exclude all natural enemies or with a net material to exclude larger predators but allow smaller parasitoids to enter the cages.

Percentage parasitism ranged from 21 to 32% in 'primera' and from 10 to 37% in 'postrera'. The nymphal density of *B. tabaci* was relatively low and ranged from 2 to 7 nymphs per leaf in 'primera' and between 0.4 to 0.9 nymphs per leaf in 'postrera'. The most common parasitoids collected from *B. tabaci* were *Encarsia pergandiella* Howard and *E. nigricephala* Dozier (Aphelinidae). Our results suggest that parasitism is host-density independent. Parasitism at low host densities (< 1 nymph per leaf) may be a contributing factor preventing *B. tabaci* outbreaks.

Key Words: Bemisia, parasitoids, predators, Zamorano

RESUMEN

Dos experimentos evaluaron el impacto de enemigos naturales sobre estados inmaduros de *Bemisia tabaci* (Gennadius) atacando dos variedades de frijol común (*Phaseolus vulgaris* L.), 'Dorado 364' (tolerante al Virus del Mosaico 'Dorado' del Frijol, VMDF) y 'Catrachita' (susceptible al VMDF). Los estudios se hicieron en la Escuela Agrícola Panamericana, Zamorano, Honduras durante la 'primera' (Mayo-Agosto) y la 'postrera' (Septiembre-Enero), 1995-96. Los tratamientos fueron dos tipos de jaulas de exclusión ($1 \times 1 \times 0.5$ m) y un tratamiento sin jaula. Las jaulas fueron cubiertas con organza, para excluir todos los enemigos naturales o con tela punto (malla), para excluir depredadores pero permitir la entrada de parasitoides.

El porcentaje de parasitismo varió entre 21 y 32% en la 'primera' y entre 10 y 37% en la 'postrera'. La densidad de ninfas fue relativamente baja y varió entre 2 y 7 ninfas por hoja en la 'primera' y entre 0.4 y 0.9 ninfas por hoja en la 'postrera'. Los parasitoides mas comunmente recolectados fueron *Encarsia pergandiella* Howard y *E. nigricephala* Dozier (Aphelinidae). Nuestros resultados sugieren que el parasitismo no está directamente relacionado con la abundancia del hospedero. El parasitismo a bajas densidades del hospedero (menos de una ninfa por hoja) puede ser un factor que contribuye a la prevención de explosiones poblacionales de *B. tabaci*.

In recent years *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) has become a major pest of beans in Honduras (Caballero 1995) and other countries of Latin America (Galvez & Morales 1989). Whitefly problems have historically occurred after the introduction of intensive cropping systems with high inputs of fertilizers and pesticides (Brown et al. 1995). This and the possible introduction of the more damaging *B. tabaci* biotype B have caused a change in pest status from sporadic to major pest in many crops including the common bean.

Bemisia tabaci is a vector of several plant viruses, e.g., bean golden mosaic virus (BGMV) and bean dwarf mosaic virus (BDMV) that reduce bean yields in Latin America (Galvez & Cardenas 1980, Brown & Bird 1995). The use of tolerant varieties and the adoption of cultural practices to reduce the likelihood of colonization by *B. tabaci* are among the few alternatives available to small scale farmers who cannot afford insecticides. Biological control of disease vectors, including *B. tabaci*, through the conservation and enhancement of native natural enemies needs to be evaluated in agroecosystems (Greathead 1991).

Information on the role of natural enemies on population dynamics of *B. tabaci* is limited to a few crops in widely separated geographic locations. In Israel, Horowitz (1986) concluded that natural enemies do not have a major effect on *B. tabaci* populations on cotton. Abiotic factors, e.g., precipitation and extreme relative humidity, assumed to cause high egg and first instar mortality, were the most important factors reducing *B. tabaci* numbers. However, Gerling (1984) had previously pointed out that the regulatory potential of parasitoids attacking *B. tabaci* would only be observed on perennial plants. In Indonesia, parasitism of *B. tabaci* ranging from 0 to 71% was observed in soybeans, but no correlation was found between parasitism and *B. tabaci* density (Kajita et al. 1992). In the same study, mortality factors other than parasitism caused reductions in *B. tabaci* numbers ranging from 0 to 78.6%. In Honduras, Velez (1993) found seven species of parasitoids attacking *B. tabaci* on common beans. *Encarsia pergandiella* Howard and *E. nigricephala* Dozier were the most common parasitoids; no correlation was observed between *B. tabaci* density and percentage parasitism.

The objective of our study was to assess the impact of biological control agents on immature stages of *B. tabaci* infesting two commercial bean varieties grown in Honduras.

Materials and Methods

Experimental Design

Field studies were carried out at the Escuela Agrícola Panamericana (EAP), located in central Honduras at approximately 800 meters above sea level. The experiments took place during the 'primera' rainy season (May-August), and the 'postrera' rainy season (September-December), 1995. Planting dates were 9 June and 7 November. Seed, fertilizer, and labor used for the establishment of the plots were provided by the Agronomy Department, EAP. Insecticides and herbicides were not used in the experiments. The total land area used was 3,000 m^2 during 'primera' and 1,600 m^2 during 'postrera'.

The bean varieties used were 'Catrachita' and 'Dorado 364', two commercial bean varieties commonly used in Honduras. 'Dorado' has a bush determinate architecture and is tolerant to the whitefly vectored bean golden mosaic virus (BGMV) (CIAT 1984). 'Catrachita' has a bush indeterminate (semi-climber) architecture, is widely used after corn in relay planting in Honduras, and is susceptible to BGMV. Treatments corresponded to two types of exclusion cages ($1 \times 1 \times 0.5$ m) and a control (no cage). The fine mesh cages (closed) were covered with organdy to exclude most insects.

The coarse mesh cages (open) were covered with a net material (approximately 70 openings per cm^2) that provided exclusion of large insects (e.g., coccinellids and chrysopids) but allowed smaller insects (e.g. aphelinids) to enter the cages. The control was a no-cage treatment that consisted of five bean plants and corresponded to the area covered by the cages (0.5 m^2 or one meter row).

For the 'primera' experiment, the two varieties were randomly assigned to main plots and the three treatments (exclusion cage types) to subplots within the main plots. Each variety and treatment was repeated four times for a total of 8 main plots and 24 subplots. Results from the 'primera' experiment showed that the exclusion of parasitoids was not being accomplished. Thus, field cages were not used in the 'postrera' experiment. In 'postrera', the two varieties were assigned randomly to each of two plots per block in a randomized block design with four repetitions.

Sampling

Field cages were set immediately after the initial whitefly infestation was detected by visual examination of ten trifoliate leaves per plot. This visual sampling was done every two days. Whitefly nymphs were first detected on 18 July. On this date ten leaves per plot were collected, taken to the laboratory and examined under the microscope to quantify initial *B. tabaci* infestation. The cages were placed in the field on 25 July and then sampled every seven days. On each sampling date, five fully-developed trifoliate leaves (one per plant) were collected in paper bags and taken to the laboratory in a cooler. Once in the laboratory, leaves were kept turgid for 3-5 days by placing each leaf petiole into a 3×10 cm glass vial containing tap water. Each glass vial was stopped with cotton and placed in a 2.5 l plastic container (one for each cage) and covered with fine mesh. After three to five days, the leaves were examined under a microscope and the number of nymphs was recorded for each leaf and cage.

The nymphs were classified into five categories based upon their appearance: 1) unparasitized (live) nymphs; 2) parasitized nymphs, in which a parasitoid larva or pupa could be observed; 3) exuviae from eclosed adult *B. tabaci*, showing a 'T' shaped opening; 4) exuviae from eclosed adult parasitoids, showing a circular opening, and 5) dry nymphs, those that seemed preyed upon or did not correspond to any other category. All nymphs corresponding to groups 1 and 2 were placed in glass vials and later dissected to confirm their status.

During 'postrera', sampling was done every seven days and started immediately after initial the whitefly infestation was detected. On each sampling date, ten fully-developed trifoliate leaves per plot (from different plants) were collected in paper bags, taken to the laboratory in a cooler and examined under a microscope. Nymphs were classified based upon their appearance into the categories described for the 'primera' experiment. Leaves infested with live or parasitized nymphs (groups 1 and 2) were kept in 2.5 liter plastic containers. After three to five days, the nymphs were checked again and dissected (when necessary) to confirm their status. Parasitized nymphs were placed in glass vials and kept at room temperature until all the parasitoids had eclosed. All collected parasitoids were cleared in lactophenol and mounted on microscope slides following procedures in Cave (1995). Parasitoids were identified by using keys in Polaszek et al. (1992). Voucher specimens of the parasitoids reared are in the insect collection of the 'Centro de Inventario Agroecológico', Crop Protection Department, EAP.

Data Analysis

The total number of nymphs per leaf was used to compute the percentages for each of the five nymphal categories for each treatment and variety on each sampling date. The percentage parasitism was calculated by adding the percentages of groups 2 (par-

asitized nymphs) and 4 (parasitoid exuviae). The percentages for each of the five groups and the total percentage parasitism for each treatment and variety were transformed using the arcsine transformation [arcsine (proportion)]. The data were analyzed under a split plot design for 'primera' and a completely randomized block design for 'postrera' using an unbalanced analysis of variance (PROC GLM, SAS Institute Inc. 1985). When appropriate, means were separated by using a least significant difference test (LSD). Percent parasitism data from 'primera' and 'postrera' and for each variety were regressed by 1) number of nymphs per leaflet and 2) number of nymphs per trifoliate, to test for host density-dependent parasitism (PROC REG, SAS Institute Inc. 1985). Mean whitefly density estimates (numbers per leaf) for each variety obtained from 18 July sample (before cage set up) were compared using a Students-t test (Ott 1993).

Results

'Primera'

On 18 July there were more *B. tabaci* nymphs per leaf on 'Dorado' (4.5 ± 0.8) (mean ± SEM) than on 'Catrachita' (2.1 ± 0.3) plots (t = 2.76; df = 4; P = 0.02). This difference, however, was not observed on the subsequent sampling dates (Fig. 1). The percentage of parasitism averaged over three sampling dates was 30.1 ± 2.0 and 24.7 ± 2.4% for Dorado and 'Catrachita', respectively. Percentage of dry nymphs (those preyed upon or not belonging to other groups) was similar for 'Dorado' (6.1 ± 1.1%) and 'Catrachita' (2.9 ± 0.6%).

Significant differences in percentage parasitism were found among treatments (cage types). The open mesh cages (those that provided partial exclusion) had lower parasitism (20.5 ± 2.5%) than the fine mesh cages and the no-cage treatment (30.2 ± 2.9 and 31.5 ± 2.4%, respectively; F = 4.32; df = 2, 12; P= 0.03) (Table 1 and Fig. 1B). The percentage of adult parasitoid exuviae increased over the sampling dates as the percentage of parasitized nymphs decreased. No differences in percentage parasitism were observed among sampling dates (Table 2).

The predominant parasitoid species collected was *E. pergandiella* (93% of the parasitoids reared, n = 126). The remaining species represented in the samples were *Encarsia porteri* (Mercet) (n = 4), *Encarsia luteola* Howard (n = 1), *E. nigricephala* (n = 1), and *Eretmocerus* sp. (n = 3). The most common predators observed on the bean plants were *Coleomegilla maculata* (DeGeer) and *Nabis* spp.; *Geocoris punctipes* (Say) was also observed on one sampling date.

'Postrera'

The population of *B. tabaci* nymphs was low throughout the 'postrera' 1995 season and never exceeded an average of one nymph per leaf (Fig. 2). No significant differences were observed between bean varieties in the average number of nymphs per leaf or the percentage parasitism (Fig. 2). Parasitism averaged over the sampling dates was 31.2 ± 5.0 % and 23.1 ± 5.2% for 'Dorado' and 'Catrachita', respectively. The percentage of dry nymphs was 9.9 ± 2.3 and 10.4 ± 3.4% for Dorado and 'Catrachita', respectively.

The average number of nymphs, and the percentages of dry nymphs and parasitoid exuviae were similar on all sampling dates (Table 3). The percentage of parasitized nymphs was lower on the last sampling date (5 January) than on all previous sampling dates except 15 December (F = 3.53; df = 4, 120) (p = 0.04). The percentage of live nymphs was significantly lower on the first two sampling dates (8 and 15 December) than on the last three sampling dates (21 and 29 December and 5 January)

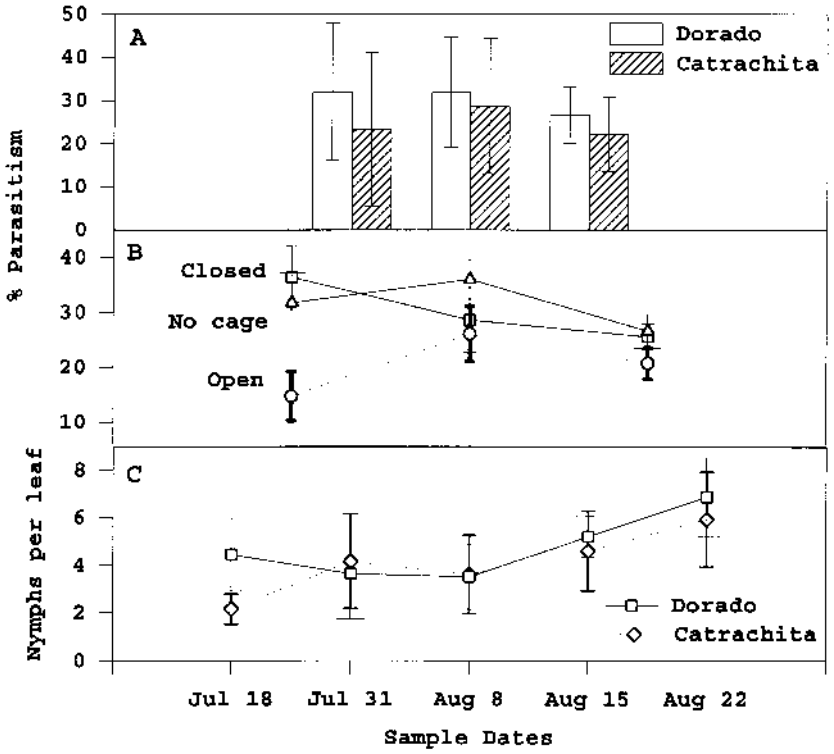

Fig. 1. Percent parasitism (± SD) of *Bemisia tabaci* nymphs in two common bean varieties (A) and two types of exclusion cages and a no cage treatment (B), and average number of nymphs per leaf (± SD) (C) during 'primera' 1995. Fine lines on error bars represent 'Dorado'.

(F = 6.75; df = 4, 12; p = 0.004) (Table 4.). In contrast, the percentage of adult *B. tabaci* eclosed exuviae was significantly higher on the first sampling date (8 December) than on the remaining sampling dates (F = 7.89; df = 4, 12) (p = 0.002) (Table 3).

Percentage parasitism did not change in response to observed fluctuations in a) number of nymphs per leaflet, b) number of nymphs per trifoliate, or c) average number of nymphs per trifoliate in either 'Dorado' or 'Catrachita' (Fig. 3A-C).

The predominant parasitoid species collected were *E. nigricephala* (n = 23) and *E. pergandiella* (n = 12) (40 and 21% of the individuals collected, respectively). The remaining species were *Encarsia hispida* De Santis (n = 11), *E. porteri* (n = 2), *E. luteola* (n = 1) and *Eretmocerus* sp. (n = 8) The most common predators observed on the bean plants were *C. maculata* and *Nabis* spp.

DISCUSSION

Parasitoids and Parasitism

Encarsia pergandiella and *E. nigricephala* were the predominant parasitoids of *B. tabaci* in our study. Both are native species and have been previously reported from

TABLE 1. AVERAGE NUMBER OF *BEMISIA TABACI* NYMPHS PER LEAF; PERCENT PARASITISM AND PERCENTAGES OF LIVE, PARASITIZED AND DRY NYMPHS, ADULT *B. TABACI* ECLOSED AND ADULT PARASITOID ECLOSED EXUVIAE ON EACH CAGE TYPE; PRIMERA 1995.

	Cage		
Variable	Fine mesh	Open mesh	No Cage
Avg. number of nymphs	4.1 ± 0.4a	3.8 ± 3.4a	4.4 ± 0.3a
Live nymphs (%)	28.5 ± 3.1a	25.9 ± 3.6a	23.8 ± 1.9a
Parasitized nymphs (%)	18.4 ± 3.0a	10.4 ± 1.6a	15.4 ± 2.7a
Adult eclosed exuviae (%)	36.9 ± 3.5a	45.5 ± 4.7a	41.1 ± 2.6a
Parasitoid exuviae (%)	11.8 ± 2.3a	10.3 ± 2.5a	16.2 ± 2.4a
Dry nymphs (%)	4.8 ± 1.4a	4.2 ± 0.1a	4.5 ± 0.1a
Percent parasitism	30.2 ± 2.9a	20.5 ± 2.5b	1.5 ± 2.4a

Numbers followed by the same letter in the same row are not statistically different ($P > 0.05$). Comparisons were made on the transformed data.

Honduras (Velez 1993) and other Latin American countries (Polaszek et al. 1992). *Encarsia pergandiella* is commonly found parasitizing *B. tabaci* on at least 14 wild host species in Honduras (Gomez 1995). In Florida, McAuslane et al. (1993) found *E. nigricephala*, *E. pergandiella*, and *Eretmocerus californicus* Howard commonly attacking *B. tabaci* on peanuts. *Encarsia nigricephala* accounted for 91% of the parasitoids col-

TABLE 2. AVERAGE NUMBER OF *BEMISIA TABACI* NYMPHS PER LEAF; PERCENT PARASITISM AND PERCENTAGES OF LIVE, PARASITIZED AND DRY NYMPHS, ADULT *B. TABACI* ECLOSED AND ADULT PARASITOID ECLOSED EXUVIAE ON EACH SAMPLING DATE; PRIMERA 1995.

	Date		
Variable	July 31	Aug. 8	Aug. 15
Avg. number of nymphs	3.9 ± 0.4ab	3.6 ± 0.3b	4.9 ± 0.3a
Live nymphs (%)	25.3 ± 2.9a	30.5 ± 3.3a	22.3 ± 2.6a
Parasitized nymphs (%)	19.0 ± 2.8a	18.1 ± 2.6a	7.2 ± 1.2b
Adult eclosed exuviae (%)	41.6 ± 3.5a	31.4 ± 3.5b	50.5 ± 3.1a
Parasitoid exuviae (%)	8.8 ± 2.2b	12.1 ± 3.0ab	17.2 ± 2.2a
Dry nymphs (%)	5.1 ± 1.4a	5.2 ± 1.1a	3.2 ± 0.7a
Percent parasitism	27.6 ± 3.5a	30.2 ± 2.9a	24.4 ± 1.6a

Numbers followed by the same letter in the same row are not statistically different ($P > 0.05$). Comparisons were made on the transformed data.

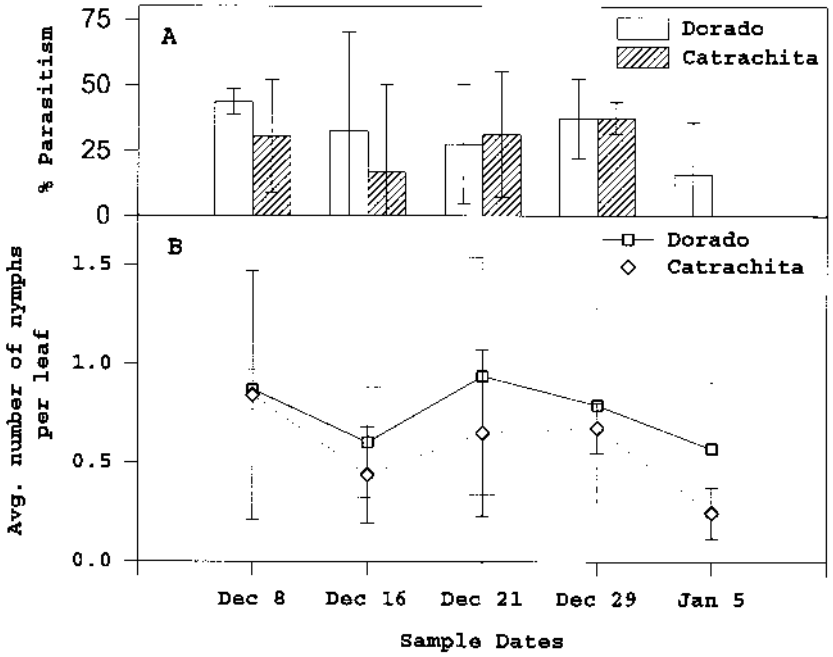

Fig. 2. A, percent parasitism (± SD) and B, average number of *Bemisia tabaci* nymphs per leaf (± SD) on common bean; 'postrera' 1995. Fine lines on error bars represent 'Dorado'.

lected in that study. The distribution and abundance of *E. pergandiella* and *E. nigricephala* reflect their importance as potential biological control agents of *B. tabaci* throughout Latin America and the southern United States.

Velez (1993) found five additional species of parasitoids attacking *B. tabaci* on common bean in Honduras, *Encarsia hispida* De Santis, *E. porteri*, *E. luteola* Howard, *Eretmocerus* sp., and *Amitus* sp. These species were also collected from 14 wild host species in Honduras (Gomez 1995). These same species except *Amitus* sp. were also reared from *B. tabaci* in our study. *Encarsia hispida* is a cosmopolitan species widely distributed in North America (Mexico, California and Florida), Central and South America, and the Caribbean (Polaszek et al. 1992). It was first synonymized with *E. meritoria* by Viggiani (1989) but later treated by Polaszek et al. (1992). Schauff et al. (1996) concluded that the original synonymization was correct and combined the two names. Similar to our study, *Encarsia porteri* accounted for less than 5% of the parasitoids reared by Velez (1993) from *B. tabaci*. Males from this species are facultative-primary egg parasitoids of various species of Lepidoptera (Rojas 1968, Arretz et al. 1985). *Encarsia luteola* is a common species and is widely distributed in the region including Mexico, Brazil, Puerto Rico, and the United States (Polaszek et al. 1992). The species of *Eretmocerus* reared in our study is a native undescribed species (M. Rose, Department of Entomology, Montana State University, personal communication).

The differences in the composition of parasitoid species collected in 'primera' and 'postrera' 1995 may be due to seasonal factors affecting each parasitoid species. Large

TABLE 3. AVERAGE NUMBER OF *BEMISIA TABACI* NYMPHS PER LEAF; PERCENT PARASITISM AND PERCENTAGES OF LIVE, PARASITIZED AND DRY NYMPHS, ADULT *B. TABACI* ECLOSED AND ADULT PARASITOID ECLOSED EXUVIAE ON EACH SAMPLING DATE; POSTRERA 1995.

	Date				
Variable	Dec. 8	Dec. 15	Dec. 21	Dec. 29	Jan. 5
Avg. No. of nymphs	0.9 ± 0.1a	0.5 ± 0.1a	0.8 ± 0.2a	0.8 ± 0.1a	0.4 ± 0.1a
Live nymphs (%)	12.4 ± 4.2b	12.5 ± 8.2b	70.5 ± 17.1a	49.1 ± 6.0a	64.6 ± 11.6a
Parasitized nymphs (%)	31.1 ± 6.2a	18.8 ± 9.7ab	29.4 ± 7.7a	36.3 ± 4.2a	7.9 ± 5.6b
Adult eclosed exuviae (%)	38.9 ± 10.3a	0.0 ± 0.0b	4.9 ± 2.6b	8.0 ± 4.1b	14.1 ± 7.2b
Parasitoid exuviae (%)	5.8 ± 4.7a	5.8 ± 4.2a	0.0 ± 0.0a	8.8 ± 8.8a	0.0 ± 0.0a
Dry nymphs (%)	11.9 ± 4.0a	1.2 ± 7.1a	4.2 ± 3.1a	3.6 ± 2.3a	13.4 ± 5.2a
Percent parasitism	36.7 ± 5.7a	24.5 ± 11.9ab	29.4 ± 7.7ab	37.2 ± 3.8a	7.9 ± 5.6b

Numbers followed by the same letter in the same row are not statistically different ($P > 0.05$).Comparisons were made on the transformed data.

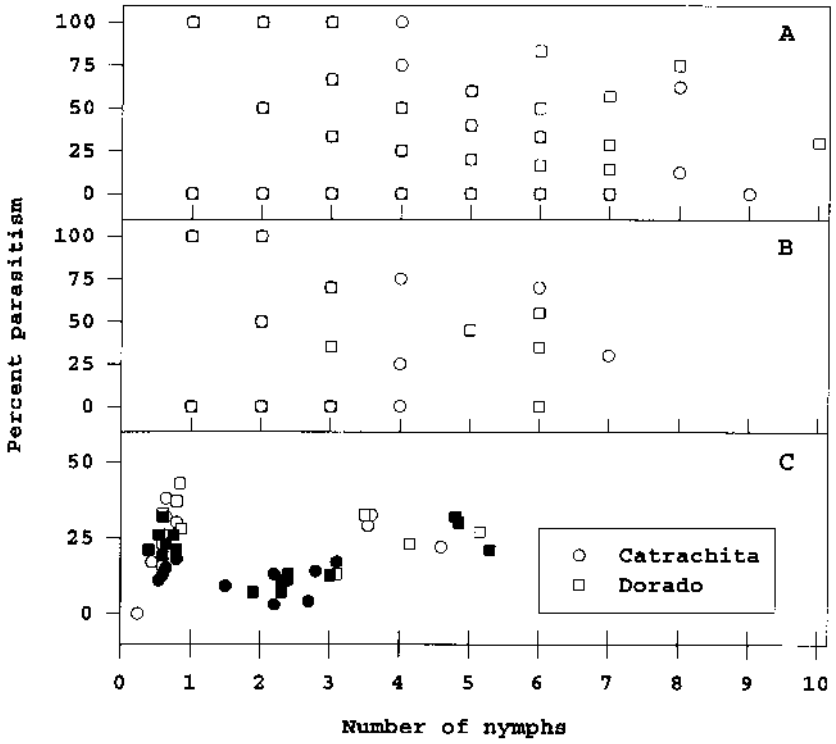

Fig. 3. Relationship between *B. tabaci* nymphal density and percent parasitism on two common bean varieties in Honduras. Square symbols represent 'Dorado'; circles represent 'Catrachita'. A, number of nymphs per leaflet, 'primera' 1995. B, number of nymphs per trifoliate leaf, 'postrera' 1995. C, average number of nymphs per trifoliate leaf, 'primera' and 'postrera', 1995 and Velez (1993). Filled symbols show data from Velez (1993) and open symbols data from this study. Note that the scale on the Y-axis is different in Fig. 3C.

variations in the composition of parasitoids species that attack *B. tabaci* have been observed between seasons at the same localities in Texas (J. Woolley, Department of Entomology, Texas A&M University, personal communication).

The differences in the percentages of the nymphal categories over time may be explained by the aging of the nymphs and parasitoids on the plants. During 'primera' 1995, there was lower parasitism in the open mesh cages compared with fine mesh cages and the no-cage treatment. These results were unexpected. The fine mesh cages did not exclude parasitoids because we were unable to cage naturally infested bean plants before parasitoid colonization. Additionally, parasitoids emerging in the fine mesh cages were unable to escape and may have parasitized the available nymphs, thus increasing levels of parasitism within the cage. The open mesh cages did not prevent parasitoids from entering or escaping, as the closed cages did, but may have deterred parasitoids from entering and attacking susceptible nymphs.

Parasitism levels in our study varied between 8 and 37%. Similar results were obtained by Velez (1993) on the same two bean varieties. These percentages are lower

than other reports from different hosts. In Egypt, parasitism of *B. tabaci* on *Lantana camara* (a perennial plant) fluctuated seasonally and reached 90% from May to October (Hafez et al. 1978). The increase in parasitism coincided with greater host densities. On cotton in California, parasitism by *Eretmocerus* sp. started at low levels but increased following the cessation of insecticide applications, reaching 70% late in the season (Bellows & Arakawa 1988). The increase in parasitism levels were also associated with an increase in *B. tabaci* numbers. These results suggest a density dependent response by the parasitoids to *B. tabaci* densities. Parasitism of *B. tabaci* fourth instars reached 90% on peanuts in Florida at the end of the season, when host densities peaked at 2.4 nymphs per leaflet (5 cm^2) (McAuslane et al. 1994). In our study, average host densities were lower than those found by MacAuslane et al. (1994) and peaked at $\cong$ 0.5 nymphs/5 cm^2 in 'primera' 1995 and $\cong$ 0.1 nymphs/5 cm^2 in 'postrera' 1995. Parasitism levels, however, remained constant over the sampling dates and seasons. This does not indicate a density dependent response by the parasitoids to these relatively low host densities.

To illustrate this point Fig. 4 shows *B. tabaci* nymphal densities and percentage parasitism observed in our study and by Velez (1993). Percentage parasitism did not change in response to changes in number of nymphs per leaflet (Fig. 3A), number of nymphs per trifoliate leaf (Fig. 3B), or the average number of nymphs per trifoliate leaf (Fig. 3C) ($r^2 < 0.01$, $P > 0.5$ in each case). Thus parasitoids find and attack a significant percentage of hosts under low host densities indicating their contribution in preventing pest outbreaks of *B. tabaci*.

Predators

The most common predators observed in this study, *C. maculata*, *Nabis* sp., and *G. punctipes*, are generalists. Mortality attributed to unknown factors and predation varied from 3 to 13%. The estimation of predation levels based on nymphal appearance may underestimate predation when predators consume whole individuals and leave no remains on the leaves, as may occur with predators with chewing mouth parts. This indicates the need to develop better methods to document predation of *B. tabaci*. Hagler et al. (1993) have developed a monoclonal antibody to test predators for consumption of *B. tabaci* and demonstrated their use in the field (Hagler & Naranjo 1994).

Biological Control of *B. tabaci* on Common Bean

Integrated pest management programs are needed that enhance the impact of native natural enemies on pest populations in the tropics (see Greathead 1991). Beans are grown under many different cropping systems in Honduras, reflecting local variations in climatic and socioeconomic factors (Woolley et al. 1991). In most of these systems, however, plant resistance can provide an economical method of disease control and will be the basis for the management of *B. tabaci* and its vectored viruses including BGMV (Galvez & Cardenas 1980). Plant resistance provides a basis for the integration of other tactics such as biological control and cultural control into integrated pest management programs. The effectiveness of cultural practices, such as changing planting dates to 'escape' high infestation levels can be enhanced by selecting short cycle tolerant varieties such as 'Dorado' (CIAT 1984). In our study, 'Dorado' appeared to have a slight advantage over 'Catrachita' in the percentage parasitism harbored under similar whitefly pressures. Even though this advantage was not statistically significant, it may be biologically important. By harboring low to moderate levels of

hosts or prey, tolerant varieties such as 'Dorado' can support natural enemy populations for enhanced suppression of *B. tabaci* populations.

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OVIPOSITION BEHAVIOR, HOST PLANT USE, AND DIET BREADTH OF *ANTHANASSA* BUTTERFLIES (LEPIDOPTERA: NYMPHALIDAE) USING PLANTS IN THE ACANTHACEAE IN A COSTA RICAN COMMUNITY

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ABSTRACT

Oviposition behavior and use of host plants by populations of *Anthanassa ardys* and *A. tulcis* (Nymphalidae: Melitaeinae) were investigated in two different habitats near Monteverde, Costa Rica. We observed oviposition behavior and collected egg clusters for experimental rearings. To explore their diet breadth, both species were reared on nine locally growing plant species in the Acanthaceae, including *Hypoestes phyllostachya*, a naturalized exotic from Africa. *A. ardys* oviposited in nature on four acanth species (*Dicliptera unguculata*, *Hypoestes phyllostachya*, *Justicia valerii*, and *Pseuderanthemum cuspidatum*) and was reared with varied success on eight species. *A. tulcis* oviposited on two acanth species (*Dicliptera unguiculata* and *Hypoestes phyllostacya*), and was reared successfully on seven. Though both species laid eggs on *Hypoestes* (5 of 14 egg clusters found), neither species successfully completed development on this plant. This oviposition "mistake" might be explained by the fact that *Anthanassa* butterflies have only recently been exposed to this plant.

Two other butterfly species, *Anartia fatima* and *Siproeta epaphus*, known to use Acanthaceae as host plants, were also unsuccessful in completing development on *Hypoestes*. Neither species was observed to oviposit on *Hypoestes* in nature.

Key Words: Acanthaceae, *Anthanassa*, exotic, *Hypoestes*, Lepidoptera, Melitaeinae, Nymphalidae, oviposition mistake

RESUMEN

Fué investigada la conducta de oviposición y el uso de plantas huésped por las poblaciones de *Anthanassa ardys* y *A. tulcis* (Nymphalidae: Melitaeinae) en dos ambientes diferentes cercanos a Monteverde, Costa Rica. Se colectaron grupos de huevos para su crianza. Con el objetivo de investigar la variedad de dietas de estas mariposas, ambas especies se criaron en nueve especies de plantas de la familia Acanthaceae, incluyendo *Hypoestes phyllostachya*, que es una planta exótica, introducida de Africa.

Las observaciones mostraron que en el campo *A. ardys* ovipositó en cuatro especies de acantáceas (*Dicliptera unguculata*, *Hypoestes phyllostachya*, con éxito en ocho especies. *A. tulcis* ovipositó en dos especies de acantáceas en el campo (*Dicliptera unguiculata* y *Hypoestes phyllostachya*), y fué criada con éxito en siete especies en el laboratorio. A pesar de que las dos especies de mariposas ovipositaron en *Hypoestes* sp. (cinco de catorce grupos encontrados), ninguna de ellas completó su crecimiento con éxito en esta planta. Esto posiblemente se deba a que *Anthanassa* spp. ovipositaron por equivocación en esta especie porque estas mariposas habían sido expuestas a esta planta recientemente.

Se conoce que otras dos especies de mariposa, *Anartia fatima* y *Siproeta epaphus*, utilizan las plantas de la familia Acanthaceae como plantas pero éstas tampoco completaron su crecimiento con éxito en el género *Hypoestes phyllostachya*. En el campo, no se observó ninguna especie que ovipositara en el género *Hypoestes*.

Anthanassa (Nymphalidae: Melitaeinae) is a genus of butterflies common in forest clearings, pastures, and disturbed open areas in tropical America. Five species of *Anthanassa* occur in Monteverde, Costa Rica: *A. ardys* (Hewitson 1864), *A. atronia* (Bates 1866), *A. crithona* (Salvin 1871), *A. otanes sopolis* (Godman & Salvin 1878) and *A. tulcis* (Bates 1864). The host plants and early stages of these butterflies are poorly known in Costa Rica and indeed in the whole neotropics (De Vries 1987). Haber observed oviposition in the field and reared *A. ardys* on *Pseuderanthemum cuspidatum* (Acanthaceae), in Monteverde (this study). Scott (1986), listing *A. tulcis* as *Phyciodes frisia* ssp. *tulcis*, cites hosts in both the Acanthaceae (*Beloperone guttata*, *Dicliptera*, *Ruellia*) and the Euphorbiaceae (*Drypetes lateriflora*). In 1997, Feldman observed *Anthanassa drusilla lelex* (Bates, 1864) ovipositing on *Justicia comata* (Acanthaceae) at La Selva Research station in Heredia, Costa Rica. We focused on the diet breadth of *A. ardys* and *A. tulcis* in Monteverde from January to March 1996 when both species were abundant.

The study site was located between 1300 and 1520 meters elevation on the Pacific slope in evergreen montane forest (Premontane Wet Forest life zone) and in areas transitional to cloud forest (Lower Montane Wet Forest life zone) (Bolaños & Watson 1993). The habitat consisted of a mosaic of primary and secondary forest patches and pasture lands. At this site the dry season begins in November, with mean monthly rainfall between 30 and 80 mm. Mean annual precipitation is 2429 mm and mean annual temperature is 19°C (Stiles & Skutch 1989).

We observed host use by *Anthanassa ardys* and *A. tulcis* at five locations spanning four km at Monteverde and determined the diet breadth for these two species by test rearings on nine species of Acanthaceae that commonly grew in the area where *Anthanassa* were active. We also wanted to determine if the exotic *Hypoestes phyllostachya* ("Polka-dot plant") served as a suitable host for any of the butterflies that use acanths at Monteverde. *Hypoestes phyllostachya* was introduced to Monteverde as an ornamental plant originating in Africa by Richard Hartmann between 1958 and 1966 (Rockwell pers. comm. 1996). In addition, we observed differences in oviposition behavior between *Anthanassa* butterflies and two other nymphalid butterflies with acanth hosts: *Anartia fatima* (Godart 1820) and *Siproeta epaphus* (Latreille 1811).

Methods

We conducted a brief survey of the acanths commonly occurring below the Monteverde Cloud Forest Preserve, and found ten species: *Blechum pyramidatum* (Lam.) Urb., *Buceragenia glandulosa* Leonard, *Dicliptera unguiculata* (Nees), *Habracanthus blepharorhachis* (Lindau) Gomez-Laur., *Hypoestes phyllostachya* Baker, *Justicia costaricana* Leonard, *Justicia oerstedii* Leonard, *Justicia valerii* Leonard, P*seuderanthemum cuspidatum* (Nees) Radlk., and *Razisea spicata* Oerst. *Buceragenia* is now considered to be a cleistogamous form of *P. cuspidatum* (M. Grayum, pers. comm.). In our study area, *Buceragenia* grew as small, frequently grazed or chopped plants in pastures, while *Pseuderanthemum* reached heights of 20 to 50 cm in shady forest edges. All ten acanths occurred in close proximity to areas where adult *Anthanassa* were abundant and active.

Anthanassa:

We observed *Anthanassa ardys* and *A. tulcis* at five locations in the Monteverde vicinity between the hours of 0900 and 1400 hr. We observed no oviposition behavior for the three other species of *Anthanassa* that occur in Monteverde: *A. atronia*, *A. cri-*

thona, and *A. otanes*. We followed females that appeared to be searching for host plants, and collected ovipositing females (whenever possible) after they stopped laying. We also noted oviposition behaviors.

From 20 January to 1 February, 1996, we collected all egg clusters found (either by observation of ovipositions or by haphazardly overturning leaves of potential acanth hosts). We witnessed seven oviposition events by *Anthanassa ardys* and two by *A. tulcis*, and collected fourteen egg clusters. Once the eggs had hatched, we transferred larvae to fresh plants and reared them in clear plastic bags in the lab, replacing fresh plant leaves every 2 or 3 days.

A. ardys

On 31 January 1996, we transferred 10 larvae from either clutches 1, 2, or 3 to each of the acanths except *Blechum*. On 3 February, we augmented these with five to ten larvae from egg cluster 6. Eleven larvae were transferred to *Blechum*. The intent was to even out the numbers of larvae on each plant as of 3 February. Total numbers of larvae placed on each plant are listed in Table 1 under "Number of Larvae." Clutches 4 and 5 died before hatching. We kept egg clusters 8, 9, and 12 on *Hypoestes*, exposing larvae to both young and mature leaves. We reared clutches 10 and 14 on *Dicliptera*, and brood 11 on *Justicia valerii*.

A. tulcis

On 4 February, we transferred four to eight larvae from brood 7 onto each of ten acanth species. We transferred 20 larvae from egg cluster 13 to *Hypoestes* including plants of varying ages, and 41 larvae from clutch 13 to *Dicliptera*.

Anartia fatima and *Siproeta epaphus*:

We observed oviposition behaviors of *A. fatima* and *S. epaphus* in the same five locations and collected eggs (laid singly) from the hostplants. On 3 February 1996, we transferred one *A. fatima* larva to *Hypoestes*, and on 9 February, we transferred one to *Dicliptera*. On 3 February, we exposed three *S. epaphus* larvae to each of these two potential hosts.

RESULTS

Oviposition Behavior

A. ardys and *A. tulcis* displayed similar oviposition behaviors. Females basked or took nectar, until around 1130 (C.S.T.), when they started flying low over clearings, roadsides, and forest edges, frequently alighting on low-growing grasses and herbaceous dicots. After landing, females appeared to test the leaves by curling their abdomens and touching them to the upper leaf surfaces. This post-landing behavior has previously been documented in other butterflies (Chew & Robbins 1984). Females did this once or twice before rejecting even a non-acanth.

Upon reaching an acceptable acanth, females alighted and rotated their bodies (either direction) in a circle on the upper surface of the leaf, touching their abdomens down many times, and beating their wings slowly and rhythmically (1-2 times per second). They performed this "dance" on the leaf surfaces for up to a minute on any one

TABLE 1. *ANTHANASSA* OVIPOSITION TIMES.

Butterfly Species Brood	Start time[2] (hrs)	Total time (min)	# of eggs	Date	Plant Species
Anthanassa ardys 1	1241	15	48	20/1/96	*Buceragenia glandulosa*[1]
Anthanassa ardys 6	1319	14	55	24/1/96	*Buceragenia glandulosa*
Anthanassa ardys 8	1220	11	67	26/1/96	*Hypoestes phyllostachya*
Anthanassa ardys 9	1236	6	38	26/1/96	*Hypoestes phyllostachya*
Anthanassa ardys 11	1202	7	55	30/1/96	*Justicia valerii*
Anthanassa ardys 14	1230	7	28	1/2/96	*Dicliptera unguiculata*
	Means:	10	48		
Anthanassa tulcis 7	1149	20	73	26/1/96	*Hypoestes phyllostachya*
Anthanassa tulcis 13	1153	19	96	1/2/96	*Dicliptera unguiculata*
	Means:	19.5	85		

Number of eggs laid by *Anthanassa ardys* was not significantly correlated with the Total time (R = 0.467, P = 0.35).

[1]*B. glandulosa* is considered to be a cleistogamous form of *P. cuspidatum*.

[2]Earliest time at which oviposition was observed.

leaf, or for several minutes of rotating on several adjacent leaves. While based only on qualitative observation, this behavior seemed to be related to the available leaf surface area on the potential hosts: if the leaf surface area was relatively large (e.g., *Pseuderanthemum*: 4-8 sq cm), the female remained on one leaf surface, but if the plant leaves were small (e.g., *Justicia valerii*: 1-3 sq cm), she moved between a few adjacent leaves and danced on them for several minutes before selecting or rejecting the plant as an oviposition site. Quantitative data is needed to test this hypothesis.

Once a leaf was selected, the female gradually stopped beating her wings while curling her abdomen onto the lower surface of the leaf, and began to oviposit. In all nine observed cases, the head and the front walking legs remained over the dorsal leaf surface. Except for the abdominal movements necessary for oviposition, the *Anthanassa* remained still, unless disturbed—in which case, rhythmic and then more rapid wing beats followed (based upon one instance where I inadvertently disturbed an ovipositing female). The earliest observed ovipositions started at 1220 hr (*A. ardys*) and 1149 hr (*A. tulcis*), and the latest began at 1319 hr (*A. ardys*) and 1153 hr (*A. tulcis*) (Table 1). Oviposition lasted 6-15 minutes in *A. ardys*, and 19-20 minutes in *A. tulcis* (Table 1). Clusters of 28-67 (*A. ardys*) and 73-96 (*A. tulcis*) eggs were laid; on one occasion two large clusters and one singly laid egg (*A. ardys*) were found on one leaf. It is not known if the clusters were from the same or different females. Eggs of *A. adys* were greenish white and bullet-shaped, with muted surface sculpturing. Eggs were

0.55mm in diameter and 0.6-0.7mm in height. Eggs of *A. tulcis* were yellow-green, spherical to bullet-shaped, and smooth with barely visible scaling on the surface. The eggs were 0.5mm in diameter and 0.4-0.5mm in height. There was no significant correlation between the duration of oviposition and the number of eggs deposited (R = 0.467, P = 0.35, Table 1—this regression was performed for *A. ardys* only). When finished ovipositing, females returned to the upper surface of the leaf and beat their wings slowly, basking for up to five minutes before flying away.

S. epaphus:

Females exhibited searching behavior similar to that of *Anthanassa*. When a female alighted on an acceptable oviposition site, she lowered her abdomen to the leaf surfaces a few times, and then extended her abdomen to lay a single egg over the course of 10-20 seconds. Females stopped beating their wings for only a few seconds while ovipositing. On the only observed host, *Blechum pyramidatum*, eggs were placed in curled leaves, the junctions of leaves and stems, or between flower bracts. Sometimes, a female returned to the same plant to deposit another egg. The eggs are spherical, 2 mm in diameter, and green with strong, vertical ribbing.

Anartia fatima:

The behavior of this butterfly was similar to that of *S. epaphus*. Single eggs (blue-green, spherical, approximately 1 mm in diameter) were laid on leaf surfaces or between flower bracts on *Blechum*, as observed at one lower-elevation site. Females also oviposited on leaf surfaces of low-growing *Hydrocotyle* sp. (Apiaceae) and *Spermacoce assurgens* (Rubiaceae), growing near small patches of *Blechum*. The five ovipositions observed lasted from 5-10 seconds each. On 22 and 24 March 1997, Feldman observed ovipositing females of *Anartia fatima* at La Selva Research Station in Heredia, Costa Rica, and found that females would land on the host (*Blechum browneii*) and take off again, subsequently ovipositing on the first plant she encountered (based on five observations of one female, one observation of another). Oviposition sites included *Blechum browneii*, *Hydrocotyle mexicana* (Apiaceae), a fern, and a dead leaf—grasses were not used as oviposition sites by these individuals.

Rearing Results

A total of 14 egg clusters were collected from 20 January to 1 February (Table 2). Six of these came from observed ovipositions of *A. ardys*, two were from observed ovipositions of *A. tulcis*, and four were found by searching acanth leaves (Table 2). All of the individuals in egg clusters 4, 5 and 12 (found by searching leaves) died before or soon after hatching, so the *Anthanassa* species were unknown for these clusters. Clusters 4 and 12 were found on *Hypoestes*, and cluster 5 was found on *J. valerii*. These data were not included in Table 2.

At least one *A. ardys* survived on each of the acanths used in this study, except for *Hypoestes* (Table 2). Although we found two egg clusters on *Hypoestes* (the two "unknown" egg masses were found on *Hypoestes*), no larvae survived past the first instar on this plant. The larvae did not appear to feed on *Hypoestes*. Although they were provided with both young and mature leaves, all larvae on *Hypoestes* died within 4-7 days. Survival rates varied among different acanth species: larvae seemed to be most successful on *Blechum*, *Dicliptera*, *Justicia* spp., and *Pseuderanthemum* (Table 2).

TABLE 2. RESULTS FROM REARING *ANTHANASSA ARDYS* AND *A. TULCIS* ON VARIOUS ACANTH SPECIES.

Butterfly Species	Cluster #	Oviposition Site Plant	Reared on	Number of Eggs[1]	Number of Larvae	Number of Adults
A. ardys[2]	1,2,3,6	*B. glandulosa*[4]	*B. pyramidatum*	146 total (48, 42, 1, 55)	10	7
" "	" " " "	" "	*B. glandulosa*		13	6
" "	" " " "	" "	*D. unguiculata*		19	6
" "	" " " "	" "	*H. blepharorhachis*		10	3
" "	" " " "	" "	*H. phyllostachya*		20	0
" "	" " " "	" "	*J. costaricana*		14	6
" "	" " " "	" "	*J. oerstedii*		20	4
" "	" " " "	" "	*J. valerii*		16	3
" "	" " " "	" "	*P. cuspidatum*		15	7
" "	" " " "	" "	*R. spicata*		10	1
A. ardys	8,9	*H. phyllostachya*	*H. phyllostachya*	67, 35	no data[5]	0
A. ardys	10	*D. unguiculata*	*D. unguiculata*	42	no data	22
A. ardys	11	*J. valerii*	*J. valerii*	55	no data	18
A. ardys	14	*D. unguiculata*	*D. unguiculata*	28	defunct[3]	
A. tulcis	7	*H. phyllostachya*	*B. pyramidatum*	73 total	7	1
" "	"	" "	*B. glandulosa*		7	2
" "	"	" "	*D. unguiculata*		7	2
" "	"	" "	*H. blepharorhachis*		4	1

[1]The "number of eggs" refers to the original number of eggs in the egg mass laid on the "Oviposition Site Plant" (host) listed on the same line in the second column. The larvae hatching out of these egg masses (the "Number of Larvae") were partitioned amongst the plants listed in the righthand column.

[2]Larvae from broods 1, 2, 3, and 6 were combined. The number of adults resulting from rearings on each host are also listed.

[3]Egg masses from which no larvae hatched are listed as "defunct."

[4]*B. glandulosa* is considered to be a cleistogamous form of *P. cuspidatum*.

[5]In these cases, larvae were not counted after the eggs hatched.

TABLE 2. (CONTINUED) RESULTS FROM REARING *ANTHANASSA ARDYS* AND *A. TULCIS* ON VARIOUS ACANTH SPECIES.

Butterfly Species	Cluster #	Oviposition Site Plant	Reared on	Number of Eggs[1]	Number of Larvae	Number of Adults
A. tulcis	"	*H. phyllostachya*	*H. phyllostachya*		7	0
" "	"	" "	*J. costaricana*		7	1
" "	"	" "	*J. oerstedii*		8	8
" "	"	" "	*J. valerii*		7	2
" "	"	" "	*P. cuspidatum*		5	2
" "	"	" "	*R. spicata*		5	0
A. tulcis	13	*D. unguiculata*	*D. unguiculata*	96 total	41	18
	"	" "	*H. phyllostachya*		20	0

[1]The "number of eggs" refers to the original number of eggs in the egg mass laid on the "Oviposition Site Plant" (host) listed on the same line in the second column. The larvae hatching out of these egg masses (the "Number of Larvae") were partitioned amongst the plants listed in the righthand column.
[2]Larvae from broods 1, 2, 3, and 6 were combined. The number of adults resulting from rearings on each host are also listed.
[3]Egg masses from which no larvae hatched are listed as "defunct."
[4]*B. glandulosa* is considered to be a cleistogamous form of *P. cuspidatum*.
[5]In these cases, larvae were not counted after the eggs hatched.

At least one *A. tulcis* survived on eight of the ten acanths used. No larvae survived past the first instar on *Razisea spicata* or *Hypoestes*, even though we observed oviposition and collected one *A. tulcis* egg cluster on *Hypoestes*. All larvae on these plants died within four days, not developing beyond the first or second instar. Again, survival rates varied among the larvae on different plants.

The one larva of *Anartia fatima* we attempted to rear on *Hypoestes* did not survive, but the one fed *Dicliptera* reached adulthood. We found no *A. fatima* eggs or larvae on *Dicliptera*. However, it is listed as a host by DeVries (1987).

When larvae of *Siproeta epaphus* were given a choice between *Hypoestes*, *Dicliptera* and *Blechum*, they fed only on *Blechum*.

Discussion

Oviposition Behaviors

Orientation toward and selection of potential oviposition sites by lepidopteran females is mediated by a combination of chemical and visual stimuli (Chew & Robbins 1984, Papaj 1986, Renwick & Chew 1994). Visual stimuli include leaf shape and color. Searching *Anthanassa* females landed on both acanths and non-acanths. Most often, the plants on which they landed were similar to the acanths in leaf shape (ovate), hinting that prior to landing on plants, these butterflies used visual cues to search for hosts.

The representatives of three genera of butterflies observed (*Anthanassa*, *Siproeta epaphus*, and *Anartia fatima*) all exhibited different oviposition behaviors. *Anthanassa* spp. invested more time for each oviposition event. Eggs were laid in clusters, which may serve as protection from predators or parasites (Haber 1978, Schmidt & Smith 1985), perhaps by reducing the surface area exposed to ovipositing parasitoids. Some lepidopteran larvae that feed in groups also have been shown to stimulate each other to feed (Chew & Robbins 1984). Females remained still during egg deposition, so unless females are tracked to the oviposition site, both laying females and egg clusters could be difficult for visually-oriented predators to find.

Siproeta laid eggs singly, relatively rapidly, and "on the move." In some instances, the host plants appeared to be too small or structurally weak to support the weight of the butterfly for more than a second or two. Eggs were laid where they were difficult to see in curled leaves, at leaf nodes, and between flower bracts. This may help hide them from visual predators. Also, many *Blechum* plants were only 5-10 cm tall, and therefore possibly too small to support more than one or two larvae.

Anartia fatima exhibited two different behaviors—one very similar to that of Siproeta. In two locations in Monteverde, females laid eggs near, but not on their host plants. Eggs were instead laid on small Apiaceae or Rubiaceae growing in close proximity to *Blechum*. This type of oviposition behavior was documented in *Anartia* (Silberglied 1983 and included references), in Ithomiinae (Haber 1978, this study), in satyrid butterflies (Singer 1984) and in Papilionidae (Young 1979). Young (1979) and Singer (1984) speculate that this strategy protects eggs from predators or parasites that search for eggs using visual or phytochemical cues of the host plants. There is evidence that some parasitoids may use phytochemicals to locate their hosts (Hendry et al. 1976).

Interspecific constraints to larval host selection in *Anthanassa* spp.

Our observations indicated no apparent partitioning of resources or microhabitats (shade or sun) between the *Anthanassa ardys* and *A. tulcis*. Both species oviposited on

Hypoestes plants in fairly close proximity and under apparently similar levels of light. Also, both species oviposited on *Dicliptera* on the same hectare of land. This could either mean that there are some unobserved differences in the microhabitats used by *A. ardys* and *A. tulcis*, or that the abundance of host plants was not limiting enough to lead to partitioning of resources.

Neither species was observed to oviposit on *Blechum* growing in the same habitat. *S. epaphus* and *A. fatima* laid eggs only on (or near) this species: they were not observed to oviposit on *Dicliptera*, *Hypoestes*, *Justicia valerii*, or *Pseuderanthemum* growing nearby. Resource partitioning between related species using different larval hosts has been documented for papilionids by Emmel & Emmel (1969) and Shapiro and Carde (1970). However, our data are insufficient to suggest competition or resource partitioning between *Anthanassa* and these other two species. Data on host use by *Anthanassa* species in other habitats and in other parts of their range are needed before reaching further conclusions.

Physiological and environmental constraints to oviposition site selection

Both *Anthanassa* species oviposited on plants along forest edges and in clearings (many acanth species grew in partial shade). Also, *Anthanassa ardys* and *A. tulcis* basked for 2-3 hours before beginning host plant searches. The shaded forest may be too cold for them. The possibility of thermoregulatory constraints to butterfly-host-plant use is discussed in Courtney (1982) and Renwick & Chew (1994). Since *Anthanassa* rarely ventured into the cooler forest, they would be less likely to encounter the shade-adapted species e.g., *Razisea spicata*, *Habracanthus blephororhachis*, *Justicia costaricana*, and *J. oerstedii*. This may explain why no oviposition was observed on these shade tolerant species. *Blechum* was most often observed growing in small patches or patches of very small plants in pastures or banana groves. It is possible that these plants were not sufficiently abundant or that resource partitioning (mentioned above) may be occurring.

Williams et al. (1996) found that females of *Phyciodes tharos* (Nymphalidae) oviposited on a range of possible hosts, while *Chlosyne harrisii* females oviposited on one host only, even though their larvae fed successfully on a range of hosts in the lab. Phytochemical as well as environmental (e.g. temperature) cues may be involved in the restricted oviposition range of *Chlosyne harrisii*. Phytochemistry plays a role in determining intraspecific differences in host plant ranges for some pierids (Huang and Renwick 1993).

Why did *A. ardys* and *A. tulcis* oviposit on *Hypoestes phyllostachya*?

Oviposition "mistakes" are documented in many lepidopteran species, such as *Anartia* and various ithomiids (Haber 1978 and pers. obs.). These "mistakes" sometimes involve females ovipositing on unrelated plants growing in close proximity to established hosts (as with *Anartia fatima*, discussed above). This has been documented by Courtney (1982), Neck (1973), and Singer (1984). Other "mistakes" involve females ovipositing on related plants that do not support larval development. These plants are often introduced species (Bowden 1971, Chew 1977 and 1981, Sevastopulo 1964, Straatman 1962).

Hypoestes phyllostachya was introduced into the Monteverde area as an ornamental plant between 1958 and 1966. The time during which *Anthanassa* spp. have been exposed to this plant species in Monteverde (a maximum of 40 years) may have been too short to allow *Anthanassa* larvae to adapt to *Hypoestes* either by rejecting this

plant as an oviposition site or by using it as a viable host. Though the visual and/or phytochemical cues are present to stimulate oviposition by *Anthanassa ardys* and *A. tulcis*, phytochemicals that stimulate feeding behavior in larvae may be absent, or phytochemicals that are present in *Hypoestes* but are absent in the native acanths may be toxic to the larvae. It appeared that the *Anthanassa* larvae did not feed on either young or mature leaves of the *Hypoestes* plants to which they were exposed. In addition, early instar larvae of *Anthanassa* spp. appear to be rather sessile, so it seems unlikely that larvae might crawl from *Hypoestes* to a normal host. Thus, it seems unlikely that this behavior in *Anthanassa* is similar to the non-host ovipositions of *Anartia fatima*.

Survival rates of *Anthanassa* spp. on various Acanthaceae

Survival rates of *Anthanassa* varied among the acanth species studied, but due to the small number of larvae reared on each plant, these findings do little more than suggest possible trends for further study. The larvae reared were most successful on *Blechum*, *Dicliptera*, *Justicia* spp., and *Pseuderanthemum* (Table 2).

Conclusions

Various factors may influence the oviposition site choices of *Anthanassa* butterflies. *Anthanassa* spp. may overlook (or simply not encounter) some potential host plant species on which they can survive (e.g., in the forest, shade or low temperatures may inhibit oviposition behavior). Alternatively, they oviposit on at least one species of Acanthaceae that is unsuitable for larval development (e.g., *Hypoestes*). However, more study is needed to determine whether acanth host plant resources are partitioned by the butterflies at Monteverde. It would also be useful to determine exactly what visual and/or phytochemical cues stimulate *Anthanassa* spp. to select oviposition sites and initiate oviposition, what chemical differences exist between *Hypoestes* and the other acanths at Monteverde, and what effects this plant might have (if any) on populations of *Anthanassa* at this site.

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FIPRONIL: AN ULTRA-LOW-DOSE BAIT TOXICANT FOR CONTROL OF RED IMPORTED FIRE ANTS (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Fipronil, a new broad spectrum pyrazole insecticide, was tested both in the laboratory and field as a bait toxicant for control of red imported fire ants, *Solenopsis invicta* Buren. Laboratory bioassays with worker ants showed that delayed toxicity occurred with baits ranging from 5 to 200 μg/ml active ingredient (AI). Tests with field-collected colonies in the laboratory confirmed the bioassay results with worker ants, and demonstrated that granular baits containing from 3.0 to 30 μg/mg (AI) eliminated colonies in 8 to 11 weeks after treatment. A field trial showed that a 15 μg/mg granular bait provided over 80% colony mortality at 6 and 12 weeks after broadcast application in non-grazed pastures. These results clearly demonstrate the potential of fipronil for use as a bait toxicant for control of red imported fire ants.

Key Words: *Solenopsis invicta*, imported fire ants, fipronil

RESUMEN

Fipronil, un insecticida pirazol nuevo de amplio espectro, fue probado en el laboratorio y en el campo en forma de cebo tóxico para el control de la hormiga importada de fuego, *Solenopsis invicta* Buren. Bioensayos de laboratorio utilizando hormigas obreras demostraron una toxicidad retrasada con cebos de 5 a 200 μg/ml de ingrediente activo (IA). Pruebas con colonias de hormigas obtenidas del campo confirmaron los resultados de los bioensayos con las hormigas obreras y demostraron que cebos granulares que contienen de 3.0 a 30 μg/mg (IA) eliminaron colonias entre 8 to 11 semanas después del tratamiento. Un experimento de campo demostró que un cebo granular de 15 μg/mg lograba más del 80% de mortalidad de la colonia entre 6 y 12 semanas después de haber sido distribuído el cebo al voleo en pasturas no en pastoreo. Estos resultados claramente demuestran el potencial de Fipronil para su uso como cebo tóxico para el control de la hormiga importada de fuego.

Most insecticides are not suitable for use as bait toxicants for control of imported fire ants (*Solenopsis invicta* Buren or *Solenopsis richteri* Forel) due to the very rigid and exacting efficacy requirements. Stringer et al. (1964) noted that an effective bait toxicant must: (1) exhibit delayed kill over at least a ten-fold dosage range and preferably above a 100-fold dosage range; (2) be rapidly transferred from one ant to another via trophallaxis and kill the recipient; and (3) not be repellent to foraging ants.

Very few insecticides possess all three of these critical characteristics, and only those that do are acceptable for use in fire ant baits. At last count, more than 7,100 compounds have been tested as fire ant bait toxicants (Banks et al. 1992). However, only six of these toxicants have ever been commercialized, and two of those are no longer marketed. Bait toxicants that are registered and available for use today in-

clude: avermectin (ASCEND™, Whitmire Laboratories, St. Louis, MO), boric acid (BUSHWHACKER, Bushwhacker Associates, Galveston, TX) two fenoxycarb products (LOGIC® and AWARD™, Novartis Crop Protection, Inc., Greensboro, NC), three hydramethylnon formulations (AMDRO® and SIEGE®, American Cyanamid, Princeton, NJ, and MAXFORCE® Ant Killer Granular Bait, The Clorox Co., Oakland, CA).

Fipronil, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazol-3-carbonitrile, is a relatively new insecticide under development worldwide by Rhone-Poulenc AG Company (Research Triangle Park, NC). This compound is a member of the family of insecticides known as phenylpyrazoles (Moffat 1993). Fipronil has demonstrated potent insecticide and acaricide properties on a large number of pests including fleas, ticks, boll weevils, thrips, flies, and others (Colliot et al. 1992, Burris et al. 1994, Postal et al. 1995, Searle et al. 1995). Oral and dermal LD_{50}s (rat) are 100 mg/kg and >2000 mg/kg, respectively (Colliot et al. 1992). We report here a series of trials in which fipronil was evaluated as a bait toxicant for control of red imported fire ants.

Materials and Methods

Laboratory Test with Field Collected Red Imported Fire Ants Workers

Fipronil was tested as a bait toxicant for control of red imported fire ants in the laboratory using techniques and procedures described by Lofgren et al. (1967). A stock solution (0.17%) was prepared by adding technical fipronil (0.233 g) to Crisco® vegetable oil (138 g). The mixture was vigorously shaken and slowly heated to approximately 43°C. This process appeared to form a super-saturated solution (most of the active ingredient [AI] was dissolved, but some particles remained in suspension). Concentrations of 1600, 800, 400, 200, 100, 25, and 5 µg/ml were then prepared from the stock solution by serial dilutions with pure Crisco vegetable oil. All dilutions did appear to form true solutions.

Each concentration, plus a nontreated check, was tested against field-collected red imported fire ants workers in the laboratory. Four replicates of each treatment were tested. Each replicate consisted of 20 workers (mix of minors and majors), confined in test chambers consisting of plastic flower pots (5 × 5 cm) that had been furnished with a Labstone® (Bayer Corp. Dental Products, South Bend, IN) bottom, which wicked moisture from an underlying bed of damp peat moss. This arrangement provided a confined area with high humidity, which is necessary for survival of the ants. The vegetable oil baits were offered to the ants by soaking cotton balls (2 mm) with each concentration prior to placing in the test chambers. The cotton balls were removed from the test chambers after a 24 hr feeding period. Mortality was assessed at 0, 1, 3, 5, 7 and 14 days after treatment.

Laboratory Tests with Field Collected Whole Colonies

Results of the laboratory bioassay with red imported fire ant workers suggested that delayed toxicity occurred with 5-200 µg/ml baits. Several trials were then conducted by preparing a series of baits within this concentration range and testing them against whole colonies rather than small groups of isolated worker ants. Baits were prepared by serially diluting stock solutions of Crisco vegetable oil containing fipronil with pure vegetable oil to form different concentrations of oil and toxicant. These solutions were then used to impregnate inert carrier granules (defatted corn grits, Illinois Cereal Mills, Paris, IL). Formulated baits contained 30% vegetable oil/toxicant

and 70% inert carrier granules (w/w). Baits containing 3, 7.5, 15, and 30 μg/mg (AI) were prepared in this manner. Each concentration was then tested against field-collected fire ant colonies in the laboratory.

Colonies were confined in plastic pails (12-liter) and allowed to acclimate in the laboratory for 5 days before testing. Water was provided as needed, but no food was offered before testing. Queen status was not determined but each colony contained several thousand workers, immatures, and alates. Formulated bait (5 g) contained in a petri dish was placed on the surface of each of 3 colonies (replicates) for each bait concentration tested. The ants were allowed to feed *ad. lib.* on the baits for 24 hours at which time the petri dishes were removed and weighed, and the amount of bait removed from the petri dish by the ants was recorded. Colonies were maintained in the laboratory under ambient temperature, watered as needed, and provided food consisting of peanut butter and live mealworms. Quantitative data were not recorded, but colonies were observed weekly for 12 weeks. Behavioral changes such as feeding, colony maintenance, and mortality were noted and recorded.

Field Trial with Fipronil Bait

Based on results from the laboratory trials, a 15 μg/mg (AI) bait was formulated by Rhone Poulenc (Research Triangle Park, NC) for more intensive testing under field conditions. The test site was non-grazed pastures located in Harrison Co., MS. All baits were applied to test plots using a shop-built granular applicator mounted on a farm tractor. The experimental design was a completely randomized design (CRD) and there were 3 replicates per treatment. Treatments included the fipronil bait which was applied at rates of 1.7 and 3.4 kg formulated bait/ha (25.5 and 51.0 mg AI/ha, respectively), a hydramethylnon standard (Amdro®, 0.73% AI bait, American Cyanamid, Princeton, NJ) applied at 1.7 kg formulated bait/ha, and a nontreated check. All test plots were 0.4 ha in size except for the fipronil plots which were 0.2 ha.

Before treatment, circular subplots with a radius of 17.9 m (0.1 ha) were established in the center of each test plot. Imported fire ant population estimates were made in each circular subplot before and 6, 12, 18, and 24 wks after pesticide application, using the population indexing system described by Harlan et al. (1981) and modified by Lofgren and Williams (1982). As shown in Table 1, this system is based on the estimated population of worker ants and the presence or absence of worker brood (larvae and pupae). Absence of worker brood suggests that a colony does not contain a normally functioning queen. A newly-formed colony with worker brood present and less than 100 workers is numerically weighted as a "5" (colony class 6). Colonies of this rating are not easily visible in the field due to their very small mound size and, thus, rarely are detected. A large mature colony with worker brood and more than 50,000 workers is assigned a weighting factor of "25" (colony class 10). The population index for a particular site is calculated as follows:

$$\text{Population Index (PI)} = \sum_{K=1}^{25} K(N_K)$$

where N_K = the number of imported fire ant colonies in a given plot with a weighting factor of K where ($25 \geq K \geq 1$). The number of active imported fire ant mounds (≥ 20 workers) and population indices were calculated for each subplot. Nontreated check plots were not treated in any manner, but were evaluated using the population estimation method previously described. These data were used to determine: 1) colony mortality, which is the percentage decrease in the pretreatment number of active mounds at each assessment interval; and 2) the percentage change in the pretreat-

TABLE 1. COLONY CLASSIFICATION SYSTEM USED TO EVALUATE THE EFFECTS OF INSECTICIDES ON IMPORTED FIRE ANT POPULATIONS.

	Worker brood absent		Worker brood present	
Number of worker ants	Colony class	Weighting factor	Colony class	Weighting factor
<100	1	1	6	5
100-1,000	2	2	7	10
1,000-10,000	3	3	8	15
10,000-50,000	4	4	9	20
>50,000	5	5	10	25

ment population indices at each assessment interval. Percentages were arc sine transformed and substitutions for values of 0% and 100% were made as stated by Gomez & Gomez (1984) before transformation. Transformed data for each treatment were separated using ANOVA and a Tukey's test (SPSS Inc. 1992).

RESULTS AND DISCUSSION

Worker Bioassay in the Laboratory

The 5 μg/ml rate did exhibit delayed toxicity, providing <25% mortality at 3 days after treatment (Fig. 1), and 97.5% mortality by 14 days after treatment. Higher concentrations (≥400 μg/ml) rendered much faster kill, approaching 100% mortality within 3 days, which is not desirable with bait toxicants as explained by Stringer et al. (1964). The intermediate concentrations (25-200 μg/ml) did not result in the rapid kill seen at higher rates, but did kill much faster than the 5 μg/ml rate.

Whole Colony Trials in the Laboratory

Granular baits containing 3, 7.5, 15.0, and 30 μg/mg (AI) were readily fed upon by test colonies in the laboratory. Some repellency was noted at 30 μg/mg because not all bait was removed by foraging workers. Delayed toxicity at each dose tested was evident by the progressive decline in number of active workers and large increase in number of cadavers. Maintenance and repair of the treated nests ceased. As an example, the routine addition of water to the nest created craters in the nest surface which were not sealed off or repaired. Check colonies performed this task within 1 to 2 hours after the craters were formed. All treated colonies died within 8 to 11 weeks after consuming the fipronil baits.

Field Trial with Fipronil Bait

The 15 μg/mg (AI) fipronil bait applied at either 1.7 or 3.4 kg formulated bait/ha (25.5 or 51.0 mg AI/ha, respectively) provided >96% reduction in pretreatment population indices at 6 and 12 weeks after application (Table 2). Colony mortality with both rates of application was >80% (Table 3). Before the 18 week evaluation, part of the property was lightly disked, and as a result, one hydramethylnon-treated plot and

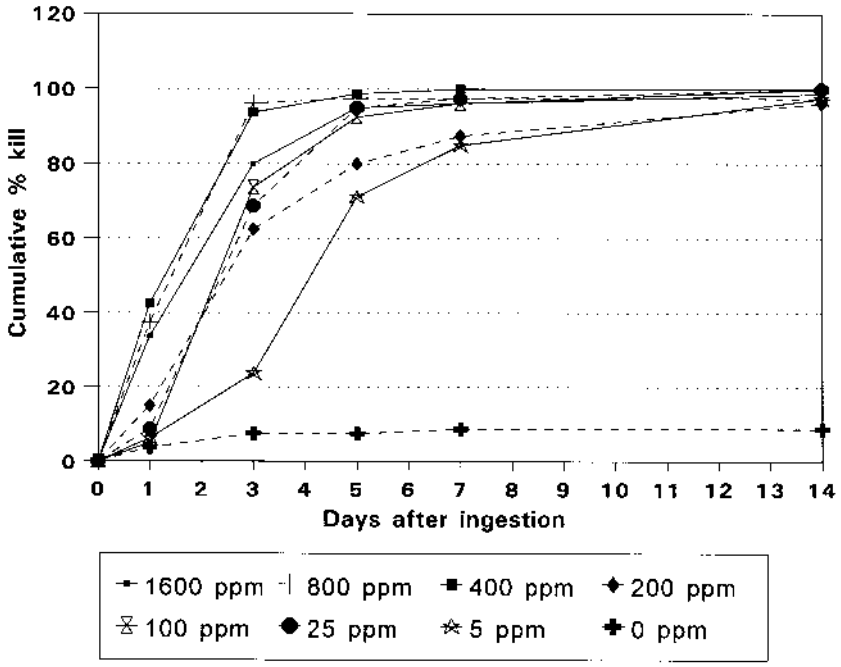

Fig. 1. Cumulative mortality to red imported fire ant workers after feeding on various concentrations of fipronil dissolved in Crisco® vegetable oil.

one nontreated check plot were lost. Therefore, treatment means for the hydramethylnon and check evaluations at 18 weeks were based on 2 replicates, rather than 3. At 18 weeks after treatment, the 1.7 kg fipronil rate provided 94.2% reduction in pretreatment population indices and 87.5% reduction in pretreatment colony numbers. The 3.4 kg rate of fipronil and the hydramethylnon standard were both reinfested with healthy colonies 18 weeks after treatment. Presence of small, incipient colonies indicated that reinfestation of all test plots had occurred. Neither fipronil rate of application was statistically different from the hydramethylnon standard at any time during this trial (Tables 2 and 3).

We have previously reported the importance of using relatively large test plots (at least 0.4 ha in size) when evaluating toxicants for control of red imported fire ants. Plots this large provide a treated buffer area of 14.2 m, which minimizes colony relocation into the test plots from adjacent nontreated areas (Collins & Callcott 1995). However, treated buffers as large as 75 m do not necessarily prevent movement from outside the treated area because Callcott & Collins (1992) noted the appearance of large, mature colonies in treated test plots with a 75 m treated buffer approximately 16 weeks after treatment. Plot size for the fipronil plots was 0.2 ha due to limited availability of the experimental bait, and therefore, these plots had treated buffers of only 4.8 m. Although overall plot size was reduced for the fipronil plots, the evaluation areas (0.1 ha) were consistent in all plots in this trial. The few remaining colonies in fipronil plots did not contain worker brood 6 and 12 weeks after treatment, indicating abnormal colonies and the apparent absence of a functioning queen. However, all of

TABLE 2. EFFECTIVENESS OF FIPRONIL BAIT AGAINST FIELD POPULATIONS OF RED IMPORTED FIRE ANTS.

Treatment	Rate of applic. (kg/ha)	Mean pop. index ± SEM - pretreat*	% change in mean population indices at indicated weeks after treatment**			
			(6)	(12)	(18)	(24)
Fipronil 0.0015%	1.7	205.0 ± 60.6	-96.6a	-96.9a	-94.2a	-67.1a
Fipronil 0.0015%	3.4	315.0 ± 18.0	-97.3a	-96.2a	-82.4a	-62.8a
Hydramethylnon 0.73%	1.7	353.3 ± 153.7	-90.1a	-88.3a	-82.4a[†]	-76.0a
Nontreated Ck	—	156.7 ± 45.1	-36.3b	-28.2b	12.0b[†]	54.4b

*Mean based on 3 replicates; see text for definition of population index method.

**Means within a column followed by the same letter are not significantly different (Tukey's test, $P \leq 0.05$; on arc sine transformed data).

[†]Before the 18 week count, one hydramethylnon replicate and one nontreated check replicate were lost due to pasture improvements, therefore only 2 replicates were included in these means.

TABLE 3. EFFECTIVENESS OF FIPRONIL BAIT AGAINST FIELD POPULATIONS OF RED IMPORTED FIRE ANTS.

Treatment	Rate of applic. (kg/ha)	Mean no. colonies present ± SEM - pretreat*	% decrease in mean no. of colonies at indicated weeks after treatment**			
			(6)	(12)	(18)	(24)
Fipronil 0.0015%	1.7	15.7 ± 5.1	81.9ab	87.5a	87.5a	61.6a
Fipronil 0.0015%	3.4	21.7 ± 2.1	84.1a	84.6a	68.6a	56.9a
Hydramethylnon 0.73%	1.7	29.3 ± 16.2	77.4ab	79.3a	79.6a[†]	76.1a
Nontreated Ck	—	12.7 ± 3.2	47.5b	36.3b	3.6b[†]	0.0b

*Mean based on 3 replicates; see text for definition of population index method.
**Means within a column followed by the same letter are not significantly different (Tukey's test, $P \leq 0.05$; on arc sine transformed data).
[†]Prior to the 18 week count, one hydramethylnon replicate and one check replicate were lost due to pasture improvements, therefore only 2 replicates were included in these means.

the plots treated at the 3.4 kg rate contained 1-4 large, normal colonies (colony class 8 according to the population index scale of Lofgren & Williams 1982) 18 weeks after treatment. While it is possible for these colonies to have developed from newly mated queens entering the treated area just after treatment, it is more likely that they migrated into test plots from the adjacent nontreated area (Markin et al. 1973, Callcott & Collins 1992). Adoption of newly mated queens by surviving broodless colonies could also account for the sudden appearance of category 8 nests within the test plots.

DISCUSSION

Laboratory tests with the new pyrazole insecticide fipronil indicated good potential for this compound as a bait toxicant against the red imported fire ant at rates of 3-30 μg/mg. The field trial showed that fipronil applied as a 15 μg/mg bait (0.0015% [AI]) at 1.7 or 3.4 kg/ha, controlled red imported fire ants as well as the hydramethylnon standard which was applied as a 0.73% (AI) bait at 1.7 kg/acre. These results indicate that fipronil met the criteria for effective fire ant bait toxicants that were listed by Stringer et al. (1964). Specifically, fipronil was effective over more than a 10-fold dosage range, was transferred throughout colonies via trophallaxis, and was not repellent to foraging workers.

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DEVELOPMENT OF *LYSIPHLEBIA JAPONICA* (HYMENOPTERA: APHIDIIDAE), A PARASITOID OF *TOXOPTERA CITRICIDA* (HOMOPTERA: APHIDIDAE) AT FIVE TEMPERATURES

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ABSTRACT

The brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy), a newly introduced pest of citrus, has become established in Florida. BrCA has shown the capacity not only to inflict direct feeding damage but also to transmit various strains of citrus tristeza virus. As a component of integrated pest management (IPM) against BrCA, an aphidiid parasitoid, *Lysiphlebia japonica* (Ashmead) was imported from Japan by USDA. In this study, the development of *L. japonica* was measured at five constant temperatures (10, 15, 20, 25, and 30°C) using BrCA as a host. Development rate from oviposition to emergence of adult wasps increased linearly with increasing temperature between 10-25°C. The developmental periods from oviposition to adult wasp emergence ranged from 29.7 d at 10°C to 9.9 d at 25°C. Developmental threshold and degree day (DD) requirement for development from oviposition to adult eclosion were 2.9°C and 223.46 DD. The percentage of parasitism varied from 49.93-23.47% within the temperature range of 10-30°C. Pupal survivorship and sex ratio decreased as temperature increased between 10-30°C. Based on our data, this parasitoid is presumably more effective in control of BrCA in cooler months than in summer months.

Key Words: *Lysiphlebia japonica*; biological control, brown citrus aphid

RESUMEN

Se ha establecido en Florida el Afido Pardo de los Cítricos *Toxoptera citricida* (Kirkaldy) (BrCA, "Brown Citrus Aphid"), que es una plaga del cítrico recientemente introducida al estado. Este áfido ha demostrado tener la capacidad no sólo para causar daño directo al alimentarse pero también para transmitir varias líneas del virus de la Tristeza de los Cítricos. Como parte de un programa de manejo integrado de plagas (IPM) contra el áfido BrCA, el parasitoide *Lysyphlebia japonica* (Ashmead) (Hymenoptera: Aphidiidae) fue importado del Japón por el USDA. En este estudio, la duración del desarrollo de *L. japonica* fué medida bajo cinco temperaturas constantes (10, 15, 20, 25 y 30°C) utilizando el BrCA como hospedero. La tasa de desarrollo desde oviposición hasta la emergencia de la avispa adulta incrementó en forma lineal con el incremento de temperaturas entre 10-25°C. La gama de la duración del desarrollo desde oviposición hasta la emergencia de la avispa adulta varió de 29.7 d a 10°C a 9.9 d a 25°C. El umbral mínimo de desarrollo y los grados-días (DD) requeridos para el desarrollo desde oviposición hasta la eclosión del adulto resultaron ser de 2.9°C y 223.46 DD. El porcentaje de parasitismo estuvo en el rango de 49.93-23.47% dentro de la gama de temperaturas de 10-30°C. La supervivencia de las pupas y la proporción sexual (macho:hembra) disminuyeron cuando se aumentó la temperatura entre 10-30°C. En base a estos datos, este parasitoide es probablemente más efectivo para el control del áfido BrCA en meses fríos que en los meses de verano.

Citrus is the most important crop in Florida, encompasses 857,687 planted acres with a total of 107 million trees in the 33 counties. The annual earnings from citrus is estimated at $1.1 billion in Florida. Citrus has many pest and disease problems, among them are the brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy) and citrus tristeza virus (CTV), which have combined as one of the most important problems for the last seven decades. Disastrous epidemics of CTV have occurred in Argentina, Brazil, Colombia, and Peru (Rocha-Pena et al. 1995). CTV was probably introduced to Florida in late 1980's from the Orient through the movement of citrus budwood plants in the quest for new citrus varieties (Roistacher & Moreno 1991, Roistacher et al. 1991).

Since the introduction of BrCA in Fall 1995 in Florida, BrCA presents a real and immediate threat to the Florida citrus industry especially to grapefruit and orange grafted on sour orange rootstock. Because it is the most efficient vector of CTV, especially for severe stem pitting strains (Costa & Grant 1951, Yokomi et al. 1994). Various control measures are being evaluated at the Institute of Food and Agricultural Sciences, University of Florida and Horticultural Research Laboratory, USDA/ARS in Orlando, FL.

One component of the IPM program against BrCA has been the importation of natural enemies. One of the parasitoids imported for control of BrCA is *Lysiphlebia japonica* (Ashmead). *L. japonica* has been recorded to parasitize several citrus aphids including *T. citricida, T. aurantii* (Boyer de Fonscolombe), *T. odinae* (Van Der Goot), *Aphis gossypii* Glover, and *A. spiraecola* Patch. in Japan and Taiwan (Stary & Schlinger 1967, Takada 1968, Kato 1970). No biological study on *L. japonica* has been conducted in the Western Hemisphere. The only report dealing with the biology of *L. japonica* was published in Japan by Takanashi (1990). Kato (1970) reported that *L. japonica* was quite effective in suppression of BrCA populations in citrus groves in Ja-

pan. We studied the effect of temperature on the development, the percentage of parasitism, pupal survivorship, and sex ratio of *L. japonica* at the five constant temperatures to evaluate the potential of *L. japonica* as a biocontrol agent of BrCA.

Materials and Methods

Host Aphid Source

Toxoptera citricida used in this study were initiated by a single collection of wild aphids from a citrus tree on the campus of Broward Community College, Davie, Broward County, Florida. The colonies were maintained on potted trifoliate citrus (*Poncirus trifoliata* (L.) Raf.) seedlings (40-50 cm tall) in an insect rearing room at 25 ± 1°C, 80 ± 5% RH and a photoperiod of 14:10 (L:D) h. After a 3-month rearing period, the ensuing colonies were used for parasitoid experiments.

Parasitoid Source

Lysiphlebia japonica used were originally imported from Nagasaki, Japan in 1996 and maintained on *Aphis spiraecola* by R. K. Yokomi at the Horticultural Research Lab, USDA/ARS in Orlando, Florida. After 11 generations of selection and breeding of *L. japonica* reared on BrCA at the Fort Lauderdale Research and Education Center, Fort Lauderdale, Florida. *L. japonica* progenies with high survivorship and reproductive rate and short life cycle on BrCA host were used for the ensuing stock colonies in an insect rearing room with conditions described above. Parasitoid adults were obtained by isolating aphid mummies singly in a small glass vial (5 by 1.5 cm diameter). Upon adult emergence, the gender was determined under a stereomicroscope. Two male adults and one female were introduced into a glass tube (11 by 0.3 cm diameter) for at least a 4-h mating period. A small piece of tissue containing 15% sugar solution was placed in each glass tube for food. The glass tube ends were covered with a piece of stretched parafilm.

Temperature Studies

About 55-65 of 2nd instar BrCA were reared on potted Duncan grapefruit (*Citrus paradisi* Macfadyen) seedlings at 2-leaf stage. A mated parasitoid female was introduced into a cage (4.2 by 1.7 cm diameter) containing 55-65 BrCA nymphs for a 24-h oviposition period at 25 ± 1°C. At least 14 potted seedlings each containing 55-65 2nd instar BrCA were used for each temperature. At the end of oviposition period, the plants with exposed nymphs were then placed in growth chambers (Percival, Boone, IA) at 10, 15, 20, 25, 30°C, 80 ± 5% RH, and a photoperiod 14:10 (L:D) h. Aphids at each temperature treatment were checked daily for presence of sedentary and bloated mummies. The mummies were collected in glass vials and returned to the same temperature treatment. All mummies were checked daily until all parasitoids emerged. The sex of adult parasitoids was determined under a stereomicroscope. Individual development time was recorded for the period from oviposition to adult emergence and from mummy formation to adult emergence.

Data Analysis

Effect of temperature on time periods from parasitoid oviposition to mummy formation, and from mummy formation to adult emergence, was analyzed by one way analysis of variance (ANOVA) and means were separated using Student-Newman-Keuls (SNK) multiple range test (GLM Procedures, SAS Institute 1985). Survival

data and the percentage of parasitism were arcine-square-root transformed before one way ANOVA and SNK multiple comparisons. A t-test was run to compare the difference of development time on male versus female within each temperature treatment. Linear regression was applied to compute the lower developmental thresholds of aphid mummies and parasitoids, using developmental rate data (1/days) as dependent variables (y-axis) and constant temperature treatments of 10-25°C as independent variables (x-axis). Development above 25°C was outside of the linear growth curve and therefore not included in the linear regression. The lower developmental threshold was determined as x-intercept of the linear equation. The degree-day (DD) required was determined as the value of the inverse of equation slope (Campbell et al. 1974). The nonlinear logistic model of Stinner et al. (1974) was used to describe the temperature-dependent development of aphid mummy and parasitoid immature: $R_t = C/(1 + \exp(k_1 + k_2 t'))$ where R_t = rate of development at temperature t, C = asymptote of the curve, k_1; k_2 = emperical constants, t' = t for t ≤ topt, t' = 2 * topt - t for > topt, and topt = temperature at which the maximum developmental rate occurs.

RESULTS

Development of Aphid Mummies

The developmental times for the mummy formation at 5 temperatures are presented in Table 1. The developmental time of mummy formation linearly decreased as temperature increased in the range of 10-25°C. However, the average developmental period at 30°C was not significantly different from the time of development at 25°C ($p < 0.05$, Table 1). A linear regression analysis comparing temperature with mummy developmental rate (10-25°C) resulted in the equation R = 0.006314 * t - 0.007122 (r^2 = 0.9986, p = 0.0007). Therefore, the mummy development of BrCA required 158.38 DD above a lower developmental threshold of 1.13°C. The nonlinear logistic model gave a good fit to the data within the range of 10-30°C. (Fig. 1) resulting in the equation: $R_t = 0.1669 /(1 + \exp(2.4130-0.1695 * t))$. When aphid host density was in the range of 55--65, the percentage of parasitism was significantly affected by temperature ($p < 0.05$, Table 1). The lowest percentage of parasitism, 23.4%, occurred at 30°C, indicating high temperatures had a detrimental effect on the parasitization rate.

TABLE 1. MEAN ± SE DEVELOPMENTAL TIME OF *T. CITRICIDA* MUMMY AND PARASITIZATION RATE AT 5 CONSTANT TEMPERATURES.

Temp. (°C)	# of mummies	Development, d	Percentage of Parasitism
10	374	17.4 ± 1.1a	49.93 ± 8.30b
15	323	11.7 ± 1.0b	47.64 ± 7.92b
20	277	8.4 ± 0.7c	49.29 ± 13.33b
25	144	6.6 ± 0.5d	61.84 ± 15.00a
30	165	6.6 ± 0.6d	23.47 ± 12.29c

Within the columns means followed by the same letters are not significantly different $p < 0.05$ (Student-Newman-Keuls multiple comparison). Percentage of parasitism data were transformed arcsine square root before Student-Newman-Keuls multiple comparison; untransformed data are presented. ANOVA statistics were: development day, F = 10660.30, df = 4,1578, $p < 0.001$; percentage of parasitism, F = 20.68, df = 4,70, $p < 0.001$.

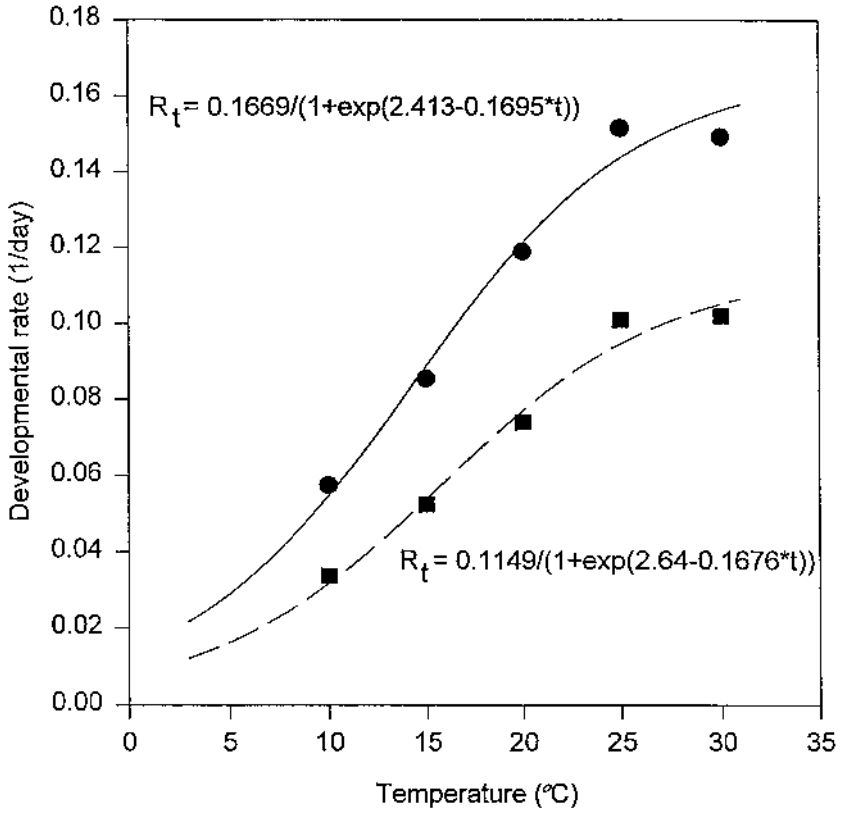

Fig. 1. Development rate (R_t) of *T. citricida* mummies (circle and solid line) and *L. japonica* (square and dotted line) at 5 constant temperatures (°C). Circles and squares are observed rates.

Development of Parasitoid

The time from oviposition to emergence of *L. japonica* was inversely correlated with the temperature in the range of 10-25°C (Table 2). An average of 29.9 ± 1.6 d and 10.1 ± 1.0 d was required for female development from oviposition to adult eclosion at 10 and 25°C, respectively. The average female developmental period at 30°C, 9.8 ± 0.6 d, was not significantly different ($p < 0.05$, Table 2) from the time of development at 25°C. The developmental times on male versus female within each temperature treatment were not significantly different (t-test, $P = 0.05$). The pooled developmental times for both sexes were also not significantly different ($p < 0.05$, Table 2) when reared on BrCA. A linear regression analysis was applied to the developmental data within the 10-25°C range. Developmental rate increased linearly with temperature, resulting in the equation $R = 0.004475 * t - 0.01303$ ($r^2 = 0.9932$, $P = 0.0034$). The theoretical developmental threshold (i.e. the point where developmental rate presumably equal 0) was estimated at 2.9°C for the male and female parasitoids. Thus, it required 223.46 DD for the parasitoid to become an adult based on this threshold. The

TABLE 2. AVERAGE NUMBER OF DAYS ± SE FROM OVIPOSITION TO ADULT EMERGENCE OF *L. JAPONICA* AT 5 CONSTANT TEMPERATURES.

Temp. (°C)	♂♂		♀♀		Pooled (♂ + ♀)	
	n	d	n	d	n	d
10	150	29.3 ± 1.5a	205	29.9 ± 1.6a	351	29.7 ± 1.5a
15	148	18.6 ± 0.9b	166	19.5 ± 1.1b	314	19.1 ± 1.1b
20	107	13.3 ± 0.6c	125	13.7 ± 0.4c	230	13.5 ± 0.8c
25	204	9.7 ± 0.6d	118	10.1 ± 1.0d	315	9.9 ± 0.5d
30	31	9.7 ± 0.5d	12	9.8 ± 0.6d	43	9.8 ± 0.5d

Within columns means followed by the same letters are not significantly different at $p > 0.05$ (Student-Newman-Keuls multiple comparison). ANOVA statistics were ♂♂, $F = 9796.46$, $df = 4,636$, $p < 0.001$; ♀♀, $F = 7326.41$, $df = 4,621$, $p < 0.001$; Pooled (♂ +♀), $F = 16132.00$, $df = 4,1262$, $p < 0.001$.

nonlinear logistic model gave a good fit to the data within the range of 10-30°C (Fig. 1), resulting in the logistic equation: $R_t = 0.1149 /(1 + \exp(2.64 - 0.1676 * t))$.

Pupal Survivorship and Adult Sex Ratio

The pupal survivorship decreased significantly as temperature increased between 20-30°C ($p < 0.05$, Table 4). The average pupal survivorship was 94.91 and 26.67% at 10 and 30°C, respectively. The average survivorship at 10°C was not significantly different from that at 15°C ($p < 0.05$, Table 4). The sex ratio was also affected by temperature, and became more male-biased as temperature increased. The values of sex ratio essentially remained the same as temperature increased within the range of 10-20°C, but change was more evident from 20 to 25 than from 25 to 30°C (Table 4).

DISCUSSION

The genus *Lysiphlebia* Stary and Schlinger is morphologically similar to the genus *Lysiphlebus* Forster (Stary & Schlinger 1967). As a result, *L. japonica* has been erro-

TABLE 3. AVERAGE NUMBER OF DAYS ± SE FROM MUMMY FORMATION TO ADULT EMERGENCE OF *L. JAPONICA* AT 5 CONSTANT TEMPERATURES.

Temp. (°C)	♂♂		♀♀		Pooled (♂ + ♀)	
	n	Days	n	Days	n	Days
10	150	11.9 ± 1.7a	205	12.5 ± 1.3a	355	12.3 ± 1.4
15	148	7.0 ± 1.0b	166	7.8 ± 1.2b	314	7.4 ± 1.2
20	107	4.9 ± 0.8c	125	5.3 ± 0.8c	232	5.1 ± 0.8
25	204	3.1 ± 0.6d	118	3.5 ± 0.4d	322	3.3 ± 0.5
30	32	3.1 ± 0.5d	12	3.2 ± 0.6d	44	3.2 ± 0.5

Within columns means followed by the same letters are not significantly different at $p > 0.05$ (Student-Newman-Keuls multiple comparison). ANOVA statistics were: ♂♂, $F = 1794.38$, $df = 4,636$, $p < 0.001$; ♀♀, $F = 1662.31$, $df = 4,621$, $p < 0.001$; Pooled (♂+♀), $F = 3834.49$, $df = 4,1262$, $p < 0.001$.

TABLE 4. PUPAL SURVIVORSHIP AND SEX RATIO OF *L. JAPONICA* AT 5 CONSTANT TEMPERATURES.

Temp. (°C)	# of mummies	# of wasps emerged	Pupal survivorship	Sex ratio (♀:♂)
10	374	355	94.9 ± 6.1a	1:0.73
15	323	314	97.2 ± 2.3a	1:0.89
20	277	232	83.8 ± 12.9b	1:0.86
25	444	322	72.5 ± 10.8c	1:1.73
30	165	44	26.7 ± 6.1d	1:2.38

Within columns means followed by the same letters are not significantly different at $p < 0.05$ (Student-Newman-Keuls multiple comparison). Survivorship data were transformed arcsine square root before Student-Newman-Keuls multiple comparison; untransformed data are presented. Pupal survivorship ANOVA: $F = 64.06$; df = 4, 70; $p < 0.001$.

neously synonyzed as *Lysiphlebus japonica* (Takanashi 1990). Overall, the developmental times from oviposition to adult emergence of *L. japonica* were shorter than those reported for *Lysiphlebus testaceipes* (Cresson) reared on *T. aurantii* at 15, 18, 21, 24, and 27°C (Tang & Yokomi 1995). Developmental times ranging 0.8-3.6 d for both males and females reared at 15, 20, and 25°C (Table 2) were shorter than those reported for both sexes at identical temperatures (Takanashi 1990). Takanashi (1990) reported that the developmental times of *L. japonica* were 21.9, 16.9, and 10.5 d for males and 22.8, 17.3, and 11.0 d for females at 15, 20, and 25°C, respectively. Although the test temperatures and host aphid species in these 2 studies were the same, the resultant developmental times were different. These differences could be attributed to differences in biotypes of host aphid and/or parasitoid. Temperature is known to differentially affect the development of host aphids and parasitoids (Campbell et al. 1974, Force & Messenger 1964). The low temperature threshold (2.9°C) of *L. japonica* was much lower than that (7.5°C) of *L. testaceipes* (Tang & Yokomi 1995). Developmental time (9.1 d at 21°C) for mummy formation of *L. testaceipes* reared on *Schizaphis graminum* (Rondani) (Hight et al. 1972) was longer than 8.4 d for *L. japonica* (Table 1).

High temperature (>25°C) had a marked negative effect on pupal survivorship of *L. japonica* (Table 4). This is in agreement with the report by Tang and Yokomi (1995) that pupal mortality of *L. testaceipes* increased greatly at 27°C and above, ranging from 24.8 to 44%. High pupal mortality of *L. japonica* at elevated temperature suggests that this parasitoid cannot tolerate the extreme temperatures in south Florida during the summer months and the seasonal diapause during hot periods as described for other aphidiid parasitoids (Stary 1988) does not occur.

One of the factors potentially affecting success or failure of released parasitoid is the offspring sex ratio (Waage & Hassell 1982). The sex ratio of parasitoids is affected by such factors as host quality, temperature, and light. The usual type of reproduction and development in parasitic Hymenoptera is for females to mate soon after emergence and to store sperm from the males in spermatheca and then, depending on external stimuli, either release sperm as eggs are being laid and produce female offspring, or to retain sperm in spermatheca so that the eggs remain unfertilized and produce male progeny (DeBach 1974). Our study showed that low temperatures (at 10 and 15°C) were a favorable external factor for *L. japonica* to produce more female progeny as compared to higher temperatures (at 25 and 30°C).

The characteristics of temperature-dependent development can be useful to conduct and evaluate biological control potential (Miller & Gerth 1994). Based on developmental periods of BrCA mummies and *L. japonica*, lower temperature threshold, pupal survival, sex ratio and parasitization rate, we suggest parasitoid release in the cooler months in south Florida. The initial recovery of *L. japonica* was only successful in the cool months (January and March) from releases during 1996-1997 reasons in the citrus groves in Davie and Ft. Pierce, Florida (Tsai unpublished) which is in agreement with the laboratory test.

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HOST-SPECIFIC ATTRACTION OF *PSEUDACTEON* FLIES (DIPTERA: PHORIDAE) TO FIRE ANT COLONIES IN BRAZIL

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ABSTRACT

Pseudacteon fly host-specificity tests were conducted in the field in southeastern Brazil with *Solenopsis* fire ants in the *saevissima* and *geminata* complexes. These parasitic flies showed a strong preference for fire ants in the *saevissima* complex. No *Pseudacteon* flies were attracted to three *Solenopsis geminata* (F.) colonies when they were set out in trays, but many flies were quickly attracted to three trays with *saevissima* complex colonies when they were set out between the *S. geminata* colonies. Even when both species of ants were placed together side by side, more than 99% of flies hovered over trays with *saevissima* complex ants. When all of the *saevissima* colonies were removed, leaving only the *S. geminata* colonies available, about 95% of flies flew away. Several flies, however, did transfer to the *S. geminata* colonies for a few minutes and at least one fly (*P. wasmanni*) attacked a few *S. geminata* workers. Altogether, 588 parasitized workers were collected from the *saevissima* complex colonies compared to 12 from the *S. geminata* colonies. Two hundred-sixty-two flies emerged from the *saevissima* complex colonies (52% *Pseudacteon tricuspis* Borgmeier, 39% *Pseudacteon litoralis* Borgmeier, 4.6% *Pseudacteon wasmanni* Schmitz, 2.7% *Pseudacteon pradei* Borgmeier, 0.4% *Pseudacteon curvatus* Borgmeier). No adult flies emerged from the *S. geminata* colonies. These results demonstrate that *P. tricuspis* and *P. litoralis* are highly specific to *saevissima* complex fire ants and strongly indicate that they would pose little threat to native fire ants should they be released as biocontrol agents for imported fire ants in the United States.

Key Words: biological control, host specificity, parasite, parasitoid, Brazil, *Solenopsis*

RESUMEN

Testes de especificidade de moscas do genero *Pseudacteon* a formigas hospedeiras foram conduzidos em condições de campo no sudeste do Brasil. Foram utilizadas formigas do genero *Solenopsis*, denominadas lava-pé, pertencentes aos complexos *saevissima* e *geminata*. Estas moscas parásitas apresentaram uma forte preferencia pelas formigas do complexo *saevissima*. Nenhuma das moscas foram atraídas pelas tres colonias de *Solenopsis geminata* (F.), quando depositadas em bandejas, entretanto, as mesmas foram rápidamente atraídas à tres bandejas contendo o complexo *saevissima* quando elas foram colocadas entre colonias de *S. geminata*. Mesmo quando ambas as especies de *Solenopsis* foram colocadas juntas, lado a lado, mais de 99% das moscas sobrevoaram a bandejas contendo o complexo de formigas *saevissima*. Após todas a colonias de *saevissima* terem sido removidas, permanecendo apenas colonias de *S. geminata*, cerca de 96% das moscas voaram, abandonando as bandejas. Várias moscas, entretanto, entraram em contato com colonias de *S. geminata* por alguns minutos e pelo menos uma mosca da especie *Pseudacteon wasmanni* Schmitz atacou algumas operarias de *S. geminata*. No total, 588 operarias parasitades foram coletadas no complexo de colonias de *saevissima*, comparado com 12 de colonias de *S. geminata*. Duzentos e sesenta e duas moscas emergiram de colonias do complexo *saevissima* (52% *Pseudacteon tricuspis* Borgmeier, 39% *Pseudacteon litoralis* Borgmeier, 4,6% *P. wasmanni*, 2,7% *Pseudacteon pradei* Borgmeier, 0,4% *Pseudacteon curvatus* Borgmeier). Nenhuma mosca adulta emergiu de colonias de *S. geminata*. Estes resultados demonstram que *P. tricuspis* e *P. litoralis* são altamente sepecíficas ao complexo de formigas lava-pé no complexo *saevissima*. Estes resultados sugerem que estas moscas parásitas apresentam pouca ameaça a formigas lava-pé nativas, se estas forem introducidas como agentes biocontroladores nos Estados Unidos.

Host specificity is an important issue that needs to be resolved before the introduction of exotic biocontrol agents. Almost 20 species of *Pseudacteon* flies in South America are known to attack species of fire ants in the *saevissima* complex of the genus *Solenopsis* (Disney 1994, Porter et al. 1995a, unpublished data). Larvae of these flies have the unusual habit of decapitating their living hosts and pupating inside the empty head capsule (Porter et al. 1995b). Field collection data indicate that most, if not all, of the species that attack fire ants are specific to fire ants (Borgmeier 1969, Borgmeier & Prado 1975, Williams & Whitcomb 1974). Field tests in Brazil also demonstrated that these flies are not attracted to ants in other genera (Porter et al. 1995a). Almost all of the flies in these tests (Porter et al. 1995a) were attracted to *saevissima* complex fire ants; however, a few flies were also attracted to fire ants in the *geminata* complex. The objective of the present study was to compare the host specificity of additional *Pseudacteon* species to *geminata* and *saevissima* complex fire ants in the field. In particular, I wanted to determine rates of attraction and successful parasitism. The suitability of *saevissima* and *geminata* complex fire ants as hosts for *Pseudacteon* flies is an important biocontrol question because all native fire ants in the United States are in the *geminata* complex (Trager 1991), while both of the imported fire ants in the United States are in the *saevissima* complex.

MATERIALS AND METHODS

Three fire ant colonies in the *saevissima* complex were collected from the EMBRAPA, CNPMA research station about 5 km south of Jaguariuna, São Paulo State, Brazil. One of these colonies was keyed to *Solenopsis invicta* Buren. The other two col-

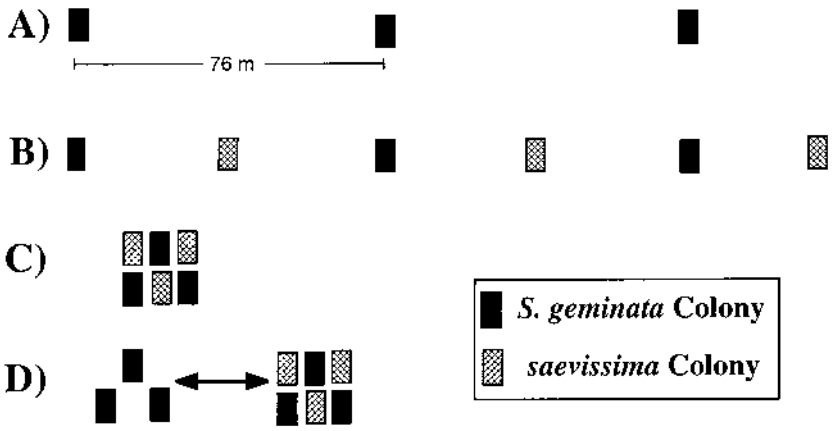

Fig. 1. Arrangement of trays with *Solenopsis geminata* and *saevissima* complex fire ants during four successive tests: A) Only *S. geminata* trays spaced 76 m apart (30 min), B) Both *S. geminata* and *saevissima* complex trays, alternately separated 38 m apart (30 min), C) Both *S. geminata* and *saevissima* trays grouped side by side at a single location (30 min), D) Alternating sequence of test *S. geminata* trays grouped together without and with the *saevissima* trays (15-20 min each test, two cycles).

onies were ambiguous between *S. invicta* and *Solenopsis saevissima* (F. Smith); both had the frontal streak, but rugous sculpture only covered half or less of the postpetiole. Three *Solenopsis geminata* (F.) colonies were collected from the CEPLAC research station about 10 km east of Itabuna, Bahia, Brazil. These colonies were the black form of *S. geminata* found in the Antilles and west Africa (Trager 1991). All test colonies lacked a mother queen and all sexuals were removed from the *S. geminata* colonies. Test colonies were placed in 40 by 26 by 8 cm nest trays. Colonies contained 3,000-10,000 workers. The sizes of the *saevissima* complex colonies were reduced to match paired *S. geminata* colonies. Field tests were conducted at two sites approximately 10 km apart to the north and east of Rio Claro, São Paulo State, Brazil along the road to Araras (8-10 April 1996).

At each site, the three *S. geminata* colonies and three *saevissima* complex colonies were set out in trays in four sequential tests as illustrated (Fig. 1). In the first test only *S. geminata* colonies were available. This was done to ensure that the *saevissima* complex ants were not diverting flies away from the *S. geminata* ants. In the second test, both kinds of ants were available at alternately spaced locations. This test was designed to show that the flies were readily attracted to their normal host (*saevissima* complex fire ants). In the third test, all six colonies were grouped together at the location having the highest fly activity. Several times during this test, the flies were shooed out of the trays and allowed to reassort themselves among the colonies. This test was designed to determine if the flies would distinguish between *S. geminata* and *saevissima* complex ants at close range after they had discovered the ants. In the fourth test, all of the *saevissima* complex colonies were removed, leaving only *S. geminata* colonies for 15-20 min. The *saevissima* colonies were then returned for about 15 min after which they were removed again leaving only the *S. geminata* colonies. This final test cycle was conducted to determine if the flies would attack *S. geminata* colonies when they were the only choice. All four tests were repeated at each site on a sec-

ond day after switching the locations of the *saevissima* complex colonies and the *S. geminata* colonies.

The numbers of active flies were estimated every 10 minutes during each test run. Flies attracted to *S. geminata* colonies were collected, identified, and quickly released. No species identifications were made for flies attracted to the *saevissima* complex colonies because of the large numbers involved. At the conclusion of these tests, all six colonies were returned to the lab and checked for pupating larvae. Flies emerging from these pupae were identified to species. *P. tricuspis* flies matched the figure in Borgmeier & Prado (1975). Voucher specimens of flies and ants have been deposited with the Museu de Zoologia, Universidade de São Paulo, Brazil; EMBRAPA's CNPMA research center in Jaguariuna, SP, Brazil, and the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Service, Division of Plant Industry, Gainesville, Florida, U.S.A.

A Fisher's exact test (Statview 4.5, Abacus Concepts, Inc., Berkeley, CA, 1995) was used for 2 by 2 contingency tables to determine if the appearance of flies over test colonies was independent of the species of ants in the colony. A Wilcoxon Signed Rank test (Statview 4.5) was used to compare the total number of flies that appeared over the three *saevissima* complex colonies with totals from the three *geminata* colonies. Totals for each species were paired by site and trial (n = 4 pairs). A non-parametric test was used because sample variance was not equal between species due to the large number of zeros associated with the *S. geminata* colonies.

RESULTS

Pseudacteon flies were not attracted to any of the *S. geminata* colonies during the first 30 min when they were the only test colonies available (Fig. 1A). Similarly, no *Pseudacteon* flies were attracted to any of the *S. geminata* colonies during the second 30 min when the *saevissima* colonies were also available (Fig. 1B). However, flies were quickly attracted to the *saevissima* complex colonies on 9 of 12 opportunities (3 colonies $\times$ 2 sites $\times$ 2 trials; 2-way contingency table, Fisher's Exact P-value = 0.0003). On average, a total of 14.3 flies were active over the *saevissima* complex colonies at each ten minute observation compared to zero over the *S. geminata* colonies (Fig. 2; Wilcoxon Signed Rank Test, P = 0.068). While not quite significant, this P-value and those following for the Wilcoxon tests are the lowest possible given the number of colonies tested.

When the six colonies were all placed together (Fig. 1C), the three *saevissima* complex colonies attracted flies on 12 of 12 possible opportunities compared to 1 of 12 for the *S. geminata* colonies (Fisher's Exact P-value < 0.0001). The average number of flies active over the three *saevissima* trays during this period was 15.4 per observation compared to 0.12 flies over the *S. geminata* trays (1 *P. litoralis* for 1 observation; it hovered but did not attack; Fig. 2; Wilcoxon Signed Rank Test, P = 0.068).

In the final test, the three *S. geminata* colonies attracted flies during 4 of 12 possible opportunities while the *saevissima* colonies attracted flies on 10 of 12 possible occasions (Fisher's Exact P-value = 0.0361). On average, 12.1 flies were active over the three *saevissima* colonies compared to an average of 0.62 flies (5 flies total) that were active when only the three *S. geminata* colonies were present (Fig. 2; Wilcoxon Signed Rank Test, P = 0.068). One *Pseudacteon wasmanni* Schmitz female was observed systematically attacking *S. geminata* workers for several minutes. The other four flies mostly hovered without attacking. When the three *saevissima* colonies were returned along side the *S. geminata* colonies, all of the flies returned almost immediately to the *saevissima* colonies. Consequently, no flies were active over adjacent *S. geminata* colonies during periods of this test cycle when both species were grouped together (Wilcoxon Signed Rank Test, P = 0.068).

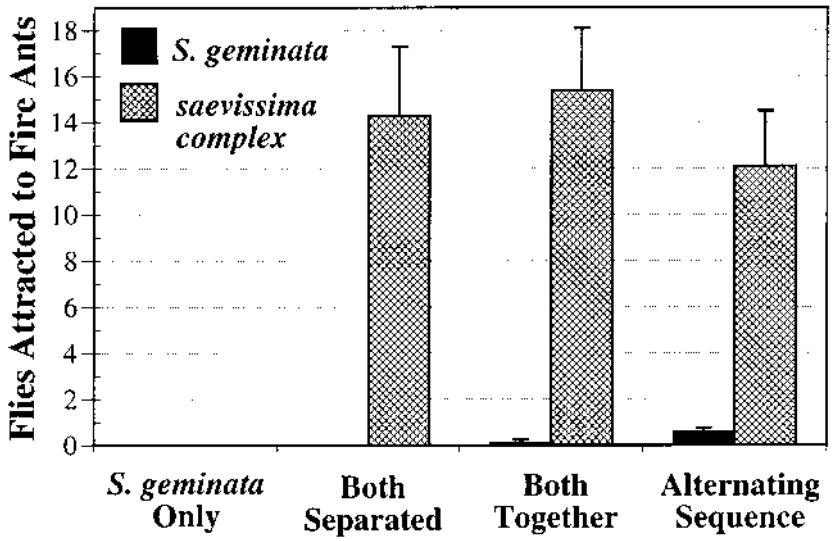

Fig. 2. Comparative abundance of parasitic *Pseudacteon* flies attracted to three *Solenopsis geminata* and three *saevissima* complex fire ant colonies in four successive time periods during which 1) only *S. geminata* colonies were available, 2) both kinds of colonies were available but separated, 3) both kinds of colonies were together side by side, 4) and both kinds of colonies were available in an alternating time sequence. Values are means of the total number of flies found in the three trays at each of four observation periods. Standard errors of the mean are indicated for each bar.

On two occasions, a dozen or so flies were placed in sealed trays with *S. geminata* fire ants so they could not escape. Most of the flies simply spent their time resting on the side of the tray or trying to escape. Two male flies hovered over the *S. geminata* workers, but no females hovered or attacked.

All six test colonies were checked for pupating fly larvae. Twelve pupating larvae were removed from two of the three *S. geminata* colonies. Four of these larvae pupated, but none emerged. Upon dissection, I identified one pupa as a female *P. wasmanni* and two as males of the same species. Although none of these flies emerged as adults, they were well developed and it seems likely that at least some *P. wasmanni* can complete development in *S. geminata.* In contrast to the small numbers of pupae in the *S. geminata* colonies, I collected 588 pupating larvae from the three *saevissima* complex colonies (320, 100, and 168, respectively). From these larvae, 262 adult flies emerged including the following five species: *Pseudacteon tricuspis* Borgmeier (52%), *Pseudacteon litoralis* Borgmeier (39%), *P. wasmanni* (4.6%), *Pseudacteon pradei* Borgmeier (2.7%), and *Pseudacteon curvatus* Borgmeier (0.4%) plus several unidentified males (1.5%). The ratio of males to females was approximately 1:1 for both *P. tricuspis* (64:71) and *P. litoralis* (54:49).

Discussion

Both *P. tricuspis* and *P. litoralis* flies showed a strong preference for the *saevissima* complex colonies over the *S. geminata* colonies. Neither species was observed attacking *S. geminata* workers, but large numbers were observed attacking the *saevissima* com-

plex colonies. Altogether, I reared 131 *P. tricuspis* flies and 102 *P. litoralis* flies from the three *saevissima* complex colonies, but none from the three *S. geminata* colonies. *P. pradei* and *P. curvatus* also were recovered only from the *saevissima* complex colonies; however, larval numbers for these two species were not sufficiently high to determine host preferences. A few *P. wasmanni* were reared from both the *saevissima* and *geminata* complex colonies. Schmitz (1914) originally reported that *P. wasmanni* attacked *S. geminata* in Joinville, Santa Catarina, Brazil; however, this report is probably in error because the nearest confirmed *S. geminata* population is in Viçosa, Minas Gerais which is more than 1000 km to the north (Fowler et al. 1995). In contrast to Porter et al. (1995a), no *P. wasmanni* or *P. pradei* females were attracted to individual *S. geminata* colonies during the initial two tests; however, one *P. wasmanni* did transfer to an *S. geminata* colony in the fourth and final set of tests. Fowler et al. (1995) reported that *P. curvatus* hovered over *S. geminata* workers from Viçosa but did not attack them.

The fact that no phorids came to the *S. geminata* colonies during the first 30 min, when they were the only fire ants available, demonstrated that most, if not all, of the *Pseudacteon* species in the two test areas had little long-range attraction to *S. geminata* fire ants. Probably, these flies simply did not recognize the odor cues produced by this fire ant even though it is in the same genus as the *saevissima* complex ants. The rapid accumulation of *Pseudacteon* flies over the *saevissima* colonies in the second 30-min period suggests that they were waiting nearby for an appropriate host.

The fact that almost no phorids attacked the *S. geminata* colonies during the third 30 min period when they were side by side with the *saevissima* colonies demonstrated that the host preferences of most flies were highly specific even at close range when flies could presumably locate their hosts visually. A high degree of host specificity was further demonstrated by the removal of the *saevissima* colonies. When the flies were given the choice of attacking *S. geminata* workers or nothing, a large majority of the flies, including all of the *P. tricuspis* and *P litoralis*, simply disappeared, choosing to attack nothing. However, when the *saevissima* colonies were returned, most of the flies returned as well, usually within 1-2 minutes. The adjacent *S. geminata* colonies were completely ignored even though the flies often had to fly directly over them to reach the *saevissima* complex ants.

In conclusion, the two most common phorids in this study (*P. tricuspis* and *P. litoralis*) both appear to have highly specific host-attraction preferences for *saevissima* complex ants (including *S. invicta*). Laboratory specificity tests with *S. geminata* and *S. invicta* from the United States also indicate highly specific preferences for *S. invicta* (Gilbert & Morrison 1997, Porter & Alonso unpublished). Considered together, these data indicate that *P. tricuspis and P. litoralis* would pose little or no risk to native *Solenopsis* fire ants.

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EFFECT OF PREY SEX, DENSITY, AND AGE ON OVIPOSITION OF *CYBOCEPHALUS* SP. NR. *NIPPONICUS* (COLEOPTERA: CYBOCEPHALIDAE), A NATURAL ENEMY OF EUONYMUS SCALE (HOMOPTERA: DIASPIDIDAE)

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ABSTRACT

Cybocephalus sp. nr. *nipponicus* Endrody-Younga (Coleoptera: Cybocephalidae) females lay their eggs individually under the cover of scale insects, similar to some hymenopteran parasitoids. Because this beetle's oviposition occurs in close association with individual scale insects, qualities of individual scale insects or patches of scale insects are factors that can potentially be used by beetles to select oviposition sites. The effect of two such factors (scale sex and density) on the oviposition of *C.* sp. nr. *nipponicus* were evaluated in the laboratory using the euonymus scale *Unaspis euonymi* (Comstock) as the ovipositional prey. For comparison, the effect of scale sex, density, and age on beetle oviposition also was investigated for a second oviposition prey, San José scale (*Quadraspidiotus perniciosus* (Comstock)).

Oviposition rates for *C.* sp. nr. *nipponicus* were strongly affected by prey sex when ovipositing on euonymus scale, with 97% of all eggs being placed under covers of male scales. In contrast, beetles placed eggs in equal proportions under covers of mature (>29 days) female and the empty scales of emerged male San José scales. Oviposition rates were affected by San José scale density. There were significantly more beetle eggs laid in patches with more than 70 scales than in those with fewer scales. Increasing scale age positively affected beetle oviposition on San José scale. Fifty-five percent of all eggs were laid in the oldest group of scales (age 53-58 days), whereas no eggs were laid in the youngest group of scales (age 9-14 days).

Key Words: *Unaspis euonymi*, biological control, *Quadraspidiotus perniciosus*

RESUMEN

Las hembras de *Cybocephalus* sp. nr. *nipponicus* Endrody-Younga (Coleoptera: Cybocephalidae) ponen sus huevecillos enforma individual debajo de las cubiertas de insectos escama de escamas, en una forma similar a algunos parasitoides himenópteros. Como existe una fuerte asociación entre este coleóptero y las escamas, las características de dichas escamas, ya sea en forma individual o en grupo, son factores que potencialmente pueden ser utilizados por los coleópteros para seleccionar sus sitios de oviposición. El efecto de dos de estos factores (sexo y densidad de las escamas) sobre la oviposición de *C.* sp. nr. *nipponicus* fueron evaluados en el laboratorio usando la escama de "euonymus," *Unaspis euonymi* (Comstock), como el huésped de oviposición. Para hacer comparaciones, los efectos del sexo, densidad, y edad de las escamas también fueron investigados sobre un segundo huésped de oviposición, la escama de San José (*Quadraspidiotus perniciosus* (Comstock)).

Cuando los coleópteros *C.* sp. nr. *nipponicus* ovipositaron en la escama de euonymus, la tasa de oviposición fué fuertemente afectada por el sexo de su huésped, con el 97% de todos los huevos siendo depositados debajo de escamas macho. En contraste, los huevos fueron puestos en igual proporción debajo de escamas hembra (>29 días) y bajo las cubiertas vacías de escamas macho cuando su huésped fue la escama de San José. Hubo un número significantemente mayor de huevos en grupos con más de 70 escamas, que en grupos con menos escamas. El aumento en la edad de las escamas de San José afectó positivamente la oviposición de los coleópteros. El 55% de todos los huevos fue ovipositado en el grupo más viejo de escamas (entre 53 y 58 días de edad), mientras que ningún huevo fue ovipositado en el grupo más joven de escamas (entre 9 y 14 días de edad).

Cybocephalus sp. nr. *nipponicus* Endrody-Younga was introduced from Korea and China into the United States as part of a USDA biological control project against euonymus scale, *Unaspis euonymi* [Comstock] (Homoptera: Diaspididae) (Drea & Hendrickson 1988). Drea & Carlson (1988) reported establishment of *C.* sp. nr. *nipponicus* at three release sites in the United States and the subsequent reduction of euonymus scale populations at these sites.

Cybocephalid beetles are predators that lay their eggs individually under the cover of diaspidid scales (Blumberg 1973, Blumberg & Swirski 1982, Nohara & Iwata 1988), similar to some hymenopteran parasitoids. Alvarez & Van Driesche (1998) studied aspects of the biology of *C.* sp. nr. *nipponicus*, which was in broad terms similar to biologies of *Cybocephalus nigriceps nigriceps* (Sahlberg), *Cybocephalus micans* Reitter, and *Cybocephalus gibbulus* (Erichson) (Blumberg & Swirski 1982; Nohara & Iwata 1988). *Cybocephalus* sp. nr. *nipponicus* females mate within two days after adult emergence and begin laying eggs beneath prey scales about four days later (Al-

varez & Van Driesche 1998). Beetles lack ovipositors; however, *C.* sp. nr. *nipponicus* females possess a telescopic abdominal segment which they use to insert eggs under a scale insect's cover. After contacting prey scales, female beetles drum scale covers and then chew a hole through the cover of the scale. Beetles then turn around and insert one or more eggs under the cover of the scale insect through the holes that the beetles cut in the cover. Sometimes eggs are inserted instead under the edge of the cover without cutting a hole. Rarely females may lay eggs under the cover of scale insects that they have previously eaten. The total number of eggs laid per female varies substantially and lifetime fertility is largely a matter of how long a female survives (Alvarez & Van Driesche 1998). Daily fertility (calculated as the mean fertility, divided by the mean longevity) (Bellows *et al*. 1992) for *C.* sp. nr. *nipponicus* is 3.11 (Alvarez & Van Driesche 1998).

Because this beetle's oviposition occurs in close association with individual prey, qualities of individual scale insects or patches of scale insects could be factors used by beetles to choose oviposition sites. The objective of this study was to clarify how *C.* sp. nr. *nipponicus* oviposition varied in response to changes in scale sex and density, using *Unaspis euonymi* as the ovipositional prey. A second ovipositional prey, San José scale, was also examined for the effect of prey sex, density, and age.

Materials and Methods

Source of Beetles and Colony Maintenance

A *C.* sp. nr. *nipponicus* colony was started at the University of Massachusetts with specimens imported from Beijing, China. Voucher specimens have been placed in the collection of the University of Massachusetts. San José scale, a natural prey species of *C.* sp. nr. *nipponicus*, reared on butternut squash (*Cucurbita* sp.), was used as prey for the beetles in the laboratory. Rearing procedures for San José scales and *C.* sp. nr *nipponicus* beetles are given in Alvarez & Van Driesche (1998).

Description of Experimental Arena and Environmental Conditions

All studies were conducted in temperature-controlled cabinets (22 ± 1°C) with a 14:10 L:D photoperiod. Because euonymus scale, the field prey of greatest interest, could not be reared apart from euonymus shrubs, naturally infested euonymus plants, *Euonymus fortunei* (L.) were collected in Amherst, Massachusetts and taken to the laboratory. Male euonymus scales were distinguished from female scales by their smaller size and distinct white color. Male scales develop a white, waxy protective covering over their bodies, while female scales develop a brown covering similar to an oyster's shell. Twig sections (10 cm long) bearing female and male euonymus scales were cut as needed for particular experiments and exposed to beetles in the laboratory. Each twig was placed in a 4-cm water vial and sealed with Parafilm®, leaving 6 cm of twig exposed. Vials with twigs were placed individually within ventilated plastic Petri dishes to confine test beetles.

A standard experimental arena was used for the experiments using San José scale. The arena consisted of 4-cm diameter circles delimited with modeling clay on test squash. Arenas were inoculated with first instar San José scale nymphs ("crawlers") as needed for particular experiments. Male San José scales were distinguished from female scales by their smaller size and distinct oblong shape of the scale covers. Adult male scales emerged approximately 20 days after crawler inoculation at 22°C. Scale covers remained intact, allowing exact counts of original numbers of scales in partic-

ular arenas, as needed for some experiments. Therefore, in these experiments, "male scales" consisted of scale covers from which an adult male had emerged. Arenas were numbered and covered with 4 cm diameter plastic Petri dishes ventilated with organdy to confine test beetles. Petri dishes were attached to the squash by pressing them into the rings of modeling clay.

Effect of Scale Sex

To assess the effect of euonymus scale sex on beetle oviposition, fifteen *C.* nr. *nipponicus* couples (one female and one male) were confined individually in ventilated plastic Petri dishes for two days. Each Petri dish contained a twig section as described previously, bearing euonymus scale females and males. After beetles were removed from the Petri dishes, euonymus twigs were examined by turning over covers of all scales to detect beetle eggs. The number of beetle eggs under covers of female and male scale insects was counted on each twig. A Chi-square test was performed to determine if beetle oviposition choice was affected by the sex of the euonymus scale preys.

To provide a comparison of *C.* nr. *nipponicus* oviposition on San José scale, mating pairs of beetles were chosen from laboratory colonies and confined in experimental arenas containing mixtures of adult male and female San José scales of standard age (25 ± 5 days). Beetles were removed from experimental arenas after two days and scale patches were examined by turning over covers of all scales to locate newly laid eggs. For every scale in the patch, we recorded the scale's sex and whether or not beetle eggs had been deposited under the scale cover. For scales with beetle eggs, the number of eggs per scale was also recorded. Sixty-nine scale patches were tested, each with a single pair of beetles. A Chi-square test was performed to examine if sex of adult San José scales affected beetle oviposition choice.

Effect of Scale Density

The effect of euonymus scale density on beetle oviposition rates (per 48 h) was examined using the same *E. fortunei* twig sections described earlier. Ten *C.* nr. *nipponicus* couples (one female, one male) were confined individually in ventilated Petri dishes on each twig. After two days, beetles were removed and the number of scale insects and the number bearing beetle eggs were counted on each twig. In addition, five pairs of beetles were placed individually as pairs, in twigs with no scales. To examine the effect of scale density on beetle oviposition a linear regression was performed.

The effect of San José scale density on beetle oviposition rates (per 48 h) was examined using the same experimental arenas described earlier and San José scale insects of a standard age (25 ± 5 days). The number of scale insects in each patch varied randomly, and was determined by the number of crawlers that successfully settled in each patch. Scales in patches consisted of mixtures of covers of emerged male scales and live adult female scales. One mating pair of beetles was chosen from laboratory colonies and confined over a patch of scale insects in an experimental arena for two days. Beetles were then removed and all scales examined. This process was replicated 70 times. In addition, ten pairs of beetles were placed individually as pairs, in arenas with no scales. Arenas with no scales included honey as food for the beetles. In arenas with scale patches, the number of scales in each patch and the number bearing beetle eggs were recorded. For scales with beetle eggs, the number of eggs per scale was recorded. Patches with scales were divided into five categories of scale density (11-40, 41-70, 71-100, 101-130, and >131) and the number of beetle eggs laid per patch was compared between groups in a one-way ANOVA. Significant differences were identified using a least significant difference (LSD) comparison test.

Effect of Scale Age

To test the effect of San José scale age on beetle oviposition, patches of scales of five different ages were exposed together in the same experimental arena. To construct a scale population with members of five different ages, each arena was divided into 5 equal wedges (72° each), and inoculated in five steps, one section every 11 days. Two days after a section was inoculated (at which time crawlers had settled), it was covered with organdy to prevent crawlers from later inoculations from entering previously inoculated sections. In this way, forty-five days after the first inoculation there were five scale patches in each arena with, respectively, scales that were 1, 12, 23, 34 or 45 days old. Following the last inoculation, scales were allowed to develop for eight more days. The experiment was replicated twenty-four times, with four replicates initiated daily over a period of six days. Each arena contained one mating pair of beetles. Ages of scales in the experiment fell into five ranges: 9 to 14 days, 20 to 25 days, 31 to 36 days, 42 to 47 days, and 53 to 58 days. Beetles were removed after two days and scale patches were examined and the number of scales per wedge and the number of beetle eggs were recorded within each age group and compared with one-way ANOVA and a least significant difference (LSD) comparison.

Results

Effect of Scale Sex

When presented with euonymus scale as prey, there were significantly more eggs laid under male scales (97.3%) than under females (2.7%) ($\chi^2 = 238.41$; $P < 0.01$). The percentages of male and female scale receiving *C.* sp. nr. *nipponicus* eggs were, respectively, 4.89 and 0.038%. The average number of eggs that a *C.* sp. nr. *nipponicus* female beetle laid on euonymus scales in a two day period was 4.9 ± 0.76 ($\bar{x} \pm$ SE; n = 15). The total numbers of female and male euonymus scales across all replications in the test were, respectively, 5283 and 1544. All the 74 eggs laid by the 15 females occurred singly.

The total numbers of female and male San José scales across all replications in the test were, respectively, 2505 and 2930. There was no difference in the total number of eggs laid in a two-day period under female and male scales (48.5% and 51.5%, respectively). Of 715 eggs laid by 69 females, 662 (92.6%) occurred singly, and 46 (6.4%) were in pairs. One group of three eggs (0.4%) and one of four eggs (0.6%) were also found. Male San José scales never received more than one egg. The average number of eggs that a *C.* sp. nr. *nipponicus* female beetle laid under San José scales in a two day period was 10.4 ± 0.8 ($\bar{x} \pm$ SE; n = 69).

The percentage of female and male San José scales receiving *C.* sp. nr. *nipponicus* eggs were, respectively, 13.9 and 12.6%. Looking only at the scales that received *C.* sp. nr. *nipponicus* eggs, the average numbers of eggs per female and per male scale were, respectively, 1.09 and 1.00. Chi-square analysis suggested that there was no relationship between beetle oviposition and the sex of the San José scale prey ($\chi^2 = 0.12$; $P = 0.7258$).

Effect of Scale Density

The average number of eggs that a *C.* sp. nr. *nipponicus* female beetle laid on euonymus scales in a two day period was 5.8 ± 1.0 (n = 10). All *C.* sp. nr. *nipponicus* eggs occurred singly and no significant relationship was observed between scale density

TABLE 1. EFFECT OF SAN JOSE SCALE DENSITY ON *CYBOCEPHALUS* SP. NR. *NIPPONICUS* OVIPOSITION IN A TWO DAY PERIOD AT 22 ± 1°C.

Scale density groups	n	Scale density Mean number (S.E.)	Oviposition Mean number (S.E.)
0	10	0.0f	0.0c
11-40	11	29.9e (2.4)	5.5b (1.9)
41-70	24	55.8d (1.9)	8.3b (1.0)
71-100	17	85.4c (2.1)	12.7a (1.9)
101-130	12	115.9b (2.5)	15.3a (2.2)
>131	6	157.2a (9.0)	15.5a (1.8)

Column values with different letters are significantly different according to one-way ANOVA and LSD criterion at the 0.05 level.

per twig and beetle oviposition. The average number of euonymus scales per twig was 493.10 ± 55.38. No eggs were laid on twigs with no scales.

The average number of eggs that a *C.* sp. nr. *nipponicus* female beetle laid on San José scales in a two day period was 10.7 ± 0.9 (n = 70). The number of eggs laid increased with scale density, although there were only two groups which differed significantly, densities over 70 scales per patch and densities below 70 (F = 4.49 df = 6,64; $P < 0.05$). The highest oviposition rate (per 48 h) (15.5 ± 1.8 eggs) was recorded for the highest scale density (Table 1). No eggs were laid in arenas with no scales.

Effect of Scale Age

The age of San José scales affected oviposition rates by *C.* sp. nr. *nipponicus* beetles. Both the number and percentage of beetle eggs laid increased with increasing scale age (Table 2). Although scale density varied slightly between treatments (because scale numbers were not standardized after inoculations), there were no statistically significant differences among numbers of scales of the different ages across the experimental arenas (F = 0.64; df = 4, 115; P = 0.637) (Table 2). Therefore, effects of scale age on beetle oviposition rates could be analyzed without adjustments for potential effects of scale density. A statistically significant difference was observed in the preference of *C.* sp. nr. *nipponicus* to oviposit in scales of different ages. Fifty-five percent of the total eggs were laid in the oldest group of scales (age 53-58 days), whereas no eggs were laid in the youngest group of scales (age 9-14 days) (F = 30.21; df = 4,115 $P < 0.05$) (Table 2).

DISCUSSION

Species of Cybocephalidae have been successfully used for the biological control of diaspididae scales (Blumberg & Swirski 1982). One of the reasons for this success could be the placement of the eggs under scale covers and the subsequent feeding by larvae under scale covers, both of which are likely to protect these stages from attack by other predators and exposure to pesticides (Alvarez & Van Driesche, 1998).

Models of reproductive behavior of predators generally assume that egg production is directly regulated by the rate of food consumption (Beddington *et al*. 1976; Gutierrez & Baumgaertner 1984), which is unlikely to be the most important factor

TABLE 2. EFFECT OF AGE OF SAN JOSÉ SCALE INSECTS ON OVIPOSITION OF *CYBOCEPHALUS* SP. NR. *NIPPONICUS* BEETLES IN A TWO DAY PERIOD AT 22 ± 1°C.

	Mean number (S.E.)		
Scale age (Days)	Oviposition (No. eggs laid/wedge)	Total scales per wedge	% of all eggs found
9-14	0.0c (0.0)	43.5a (4.4)	0.0c (0.0)
20-25	1.1bc (2.2)	45.0a (0.1)	8.8bc (3.0)
31-36	1.9b (1.8)	41.0a (4.1)	18.1b (3.9)
42-47	2.5b (3.5)	40.3a (4.6)	18.3b (4.2)
53-58	5.3a (3.4)	50.5a (5.8)	54.8a (5.5)

Column values with separate letters were significantly different in a one-way ANOVA with an LSD criterion at the 0.05 level.

governing the oviposition strategy of *Cybocephalus* nr. *nipponicus* beetles. Availability of food is not the only factor involved in egg production and oviposition of *C.* nr *nipponicus*. Female beetles were able to respond to the absence of prey, withholding eggs for two days, even though they were supplied with honey and water as food sources. Therefore, oviposition is likely in part to be triggered by certain qualitative features of scale populations. Ladybird beetles have been shown to respond to cues indicating the presence of their prey (i.e., aphids). Some female coccinelids can withhold eggs for several hours in the absence of such cues (Evans & Dixon 1986). It is possible that the survival of *C.* nr. *nipponicus* in the field depends on the presence of scale prey at the time of oviposition.

Also, as demonstrated by Mills (1982) with ladybird beetles, *C.* nr. *nipponicus* females responded to increases in scale density abundance by increasing oviposition up to a maximum (71 scales per experimental arena). At still higher scale densities, the rate of egg production is constant and independent of prey abundance. Unlike coccinellids, cybocephalids are able to maintain their populations at low scale densities and thus help keep scale populations below damaging levels (Alvarez & Van Driesche 1998).

In some cases, *C.* nr. *nipponicus* eggs were found in groups under scale covers. The number of eggs laid by females under a given scale may depend on several factors, including the number of available scales in a patch. It seems that if females do not find new scales to oviposit under, they lay their eggs under scales on which oviposition has already occurred.

Oviposition rates for *C.* sp. nr. *nipponicus* beetles were strongly affected by the sex of the prey when oviposition occurred on euonymus scale, with nearly all eggs being laid under male scales. In contrast, beetles placed eggs in equal proportions under female and male San José scales. While cover thickness was not measured in these experiments, difference in cover thickness between these two scale species and their sexes could explain this oviposition preference. Female and male of San José scale are much more similar in size and thickness of covers than are the different sexes of euonymus scale (Alvarez, unpublished data). Honda and Luck (1995) noted that the physical characteristics of the scale cover affects the successful suppression of diaspidid scales by coccinellid predators.

When presented with scales of different ages in one patch, *C.* nr. *nipponicus* preferred to oviposit under the oldest scales. Alvarez & Van Driesche (1998) showed pre-

viously that beetle larvae need to consume more prey if feeding on younger scales than if feeding on older scales. Furthermore, the highest larval survival (to the adult) occurred when larvae fed on scales older than 30 days (Alvarez & Van Driesche 1998). Oviposition under covers of older scales may therefore suggest that the survival of beetle offspring depends on the placement of the eggs under scales best able to provide a suitable food resource to beetle larvae, resulting in enhanced larval survival. As noted by Blumberg & Swirski (1982) when working with *Cybocephalus micans* Reitter and *C. nigriceps nigriceps* (Sahlberg), *C.* nr. *nipponicus* eggs were found to be laid under bodies of dead female scales only rarely. The causal mechanism, which affects beetle selection of live, older scales, remains unknown.

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LIFE HISTORY OF *BEMISIA ARGENTIFOLII* (HOMOPTERA: ALEYRODIDAE) ON *HIBISCUS ROSA-SINENSIS* (MALVACEAE)

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ABSTRACT

Oviposition, development and survivorship of *Bemisia argentifolii* Bellows & Perring, were evaluated on two cultivars of hibiscus (*Hibiscus rosa-sinensis* L.), 'Brilliant Red' and 'Pink Versicolor'. *B. argentifolii* deposited significantly more eggs and survivorship of nymphs was significantly greater in choice tests on 'Pink Versicolor' than on 'Brilliant Red'. Overall developmental time of *B. argentifolii* from egg to adult eclosion was longer on 'Brilliant Red' than on 'Pink Versicolor'. Although not all differences between the two cultivars were statistically significant, 'Brilliant Red' could be considered a less favorable host for silverleaf whitefly than 'Pink Versicolor'. However, based on the intrinsic rate of increase (r = 0.105) and finite rate of increase (λ = 1.22) of *B. argentifolii*, even 'Pink Versicolor' was a poor host for *B. argentifolii* compared to published values for eggplant, tomato, sweet potato, cucumber, garden bean, or collard.

Key Words: silverleaf whitefly, sweetpotato whitefly, oviposition, development, hibiscus, greenhouse, rate of increase

RESUMEN

Se evaluaron las tasas de oviposición, desarrollo y sobrevivencia de *Bemisia argentifolii* Bellows & Perring en dos variedades de tulipán (*Hibiscus rosa-sinensis* L.), 'Brilliant Red' y 'Pink Versicolor'. En pruebas de elección, *B. argentifolii* depositó significativamente más huevecillos sobre 'Pink Versicolor' que sobre 'Brilliant Red', y la tasa de sobrevivencia de ninfas fue significativamente mayor en la primer variedad que en la segunda. El desarrollo del huevecillo hasta la eclosión del adulto duró más en la variedad del tulipán 'Brilliant Red' que en 'Pink Versicolor', a pesar de que las diferencias no eran siempre significativas entre estadíos. Aunque las diferencias entre las dos variedades de tulipán no fueron significativas, se puede considerar que la variedad 'Brilliant Red' es un hospedero menos favorable para el desarrollo de esta especie de mosca blanca que 'Pink Versicolor'. Sin embargo, tampoco 'Pink Versicolor' actuó como un huésped óptimo para el desarrollo de *B. argentifolii* según el criterio de la tasas de incremento intrínseco y finito (r = 0.105 y λ = 1.22 respectivamente), en comparación con los valores publicados para su desarrollo en berenjena, tomate, camote, pepino, frijol o col rizada.

Bemisia argentifolii Bellows & Perring, formerly known as sweetpotato whitefly, *B. tabaci* (Gennadius) Strain 'B', was recognized as a greenhouse pest of ornamentals (poinsettia, *Euphorbia pulcherrima* Willd.) (Hamon and Salguero 1986, Price et al. 1986) even before becoming a major pest of field crops and vegetables (Perring *et al*.,

1993). Ornamental plants infested with whiteflies risk rejection in both domestic and international markets (Alderman 1987, Schuster et al. 1996), and even the presence of empty exuviae is considered unacceptable (N. Rechcigl, Yoder Brothers, personal communication). These marketing realities exacerbate the impact of *B. argentifolii* on ornamental hosts including hibiscus (*Hibiscus rosa-sinensis* L.).

Development of management systems for *B. argentifolii* has been aided by biological studies in field crops (Avidov 1956, Azab et al. 1971, Butler et al. 1983, Bethke et al. 1991, von Arx et al. 1983), vegetables (Sharaf 1984, Tsai and Wang 1996), and some ornamental plants (Enkegaard 1993, Fransen 1990, Oetting and Buntin 1996), but not hibiscus. Host plant resistance might be a viable management strategy for hibiscus, but no sources of resistant germplasm have been identified. However, it is commonly believed that whiteflies are more problematic on cultivars with light-colored flowers and foliage than the traditional red flowered dark foliage varieties (N. Rechcigl, Yoder Brothers, Inc., personal communication). In a preference study of 12 hibiscus cultivars in Texas, Meagher and Estrada (1994) found that 'Cooper II', 'Ross Estey' and 'Dainty White' had the lowest numbers of eggs and nymphs, and 'Joanne', 'Gold Dust', 'Butterfly', 'Mary Morgan', and several others had the greatest population 6 weeks after initial infestation with *B. argentifolii*.

The objectives of this study were: (1) to compare whitefly oviposition and development on two most common cultivars of hibiscus in Florida, (2) to evaluate additional life history characteristics on one of these cultivars, and (3) to use this information to suggest ways of integrating host plant resistance and biological control into current management practices.

Materials and Methods

Host Plants and Whitefly

Two cultivars of hibiscus (*Hibiscus rosa-sinensis* L.) were used, 'Brilliant Red' (red blooms, dark green foliage and considered whitefly tolerant), and 'Pink Versicolor' (pink blooms, lighter foliage and considered susceptible). All plant materials were provided by Yoder Brothers Inc., Parish, FL. Cuttings (15-cm high) were individually planted in 15-cm plastic pots using standard media (Metro-Mix 300 growing medium, Grace Sierra, Horticultural Products Company, Milpitas, CA). *B. argentifolii* have been maintained on a mixture of several host plants: collard (*Brassica oleracea* L. var. *acephala*, 'Georgia LS'), tomato (*Lycopersicum esculentum* Miller, 'Lanai'), and sweet potato (*Ipomoea batatas* L., 'Carolina Bunch') in an air-conditioned greenhouse for >5 years, and were identified as strain 'B' of sweetpotato whitefly, *B. tabaci* (Gennadius), before the new species name was designated.

Feeding and Oviposition Preference

Choice and non-choice tests were conducted to compare the feeding and oviposition preference of *B. argentifolii* adults on the two hibiscus cultivars. Eight plants for each cultivar were selected, and 3 leaves from the top of the plant of similar age were removed from each plant, leaving a total of 24 leaves from each cultivar, or 48 leaves total. Six leaves, 3 from each cultivar, were randomly selected, labeled with India ink, and inserted in one of 6, 20-ml glass vials which had been taped together to form a "leaf-wheel" as described in Liu and Stansly (1995). The leaf wheels were individually placed into cages ($60 \times 60 \times 60$ cm) into which 120 adult females of *B. argentifolii* (20 adult females per leaf) were introduced. Numbers of *B. argentifolii* adult females and

eggs on each leaf were recorded 24 h later. Leaf area was measured using a leaf area meter (Li-Cor 2000, Lincoln, NE) and data expressed as adult females and eggs per cm^2 leaf area. The experiment included 8 replicates (leaf wheels) for a total of 48 leaves. Six leaves from each of the same cultivar were collected and arranged in each leaf wheel for the no-choice control.

Development and Survival of Immatures

Hibiscus cuttings (15-20 cm high) of 'Pink Versicolor' and 'Brilliant Red' were individually transplanted in plastic pots (15-cm in diameter) filled with Metro-Mix 300 growing medium. Plants were watered with 0.4% (wt.:vol.) Stern's Miracle-Gro (an all purpose water soluble plant food with N-P-K: 15-30-15) (Stern's Miracle-Gro Products, Port Washington, NY) twice per week and grown to approximately 40 cm high with 4-5 fully expanded leaves. Old leaves were removed so that only the two top fully expanded leaves remained. *B. argentifolii* adults (60 unsexed) collected from the greenhouse colony, were introduced into clip-on cages (4 cm in diameter) for oviposition, and were removed 2 h later to optimize uniformity of subsequent stages. Indian ink was used to place an identifying mark next to 20 whitefly eggs on each of 8 leaves per cultivar. Infested plants were placed in 60 × 60 × 60 cm cages and development and survivorship of each whitefly was recorded daily until all live whiteflies had emerged.

Life Table

Bemisia argentifolii pupae were collected from an insectary colony on 'Pink Versicolor'. Small confinement cages were made from gelatin capsules (No. 000, Torpac, Fairfield, NJ) on which the closed end of the narrow half was removed to form a tube which was held in place on the leaf by a piece of double-stick cellophane tape (15 × 15 × 2 mm, Double-sided Mounting Tape, Ace Hardware, Oak Brook, IL) into which a capsule-sized hole had been punched. The wider capsule half was used as the cage cover (Fig. 1). A cage was placed on the abaxial surface of the top fully expanded young leaf of a potted 'Pink Versicolor' plant (45-50 cm high). A pair of male and female whitefly adults having emerged 14 h earlier was introduced into the gelatin capsule

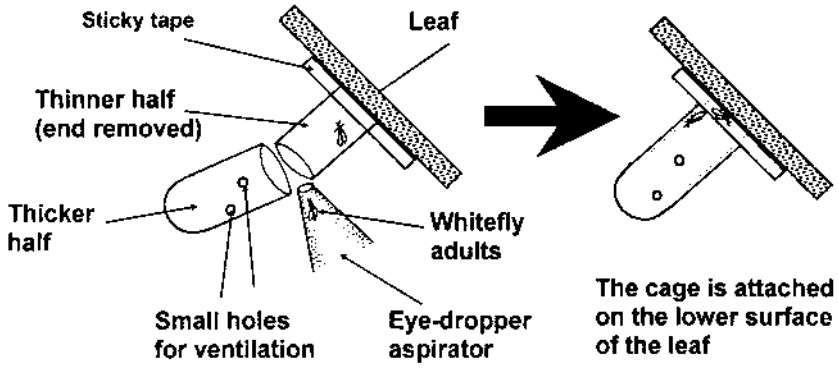

Fig. 1. The gelatin capsule cage and the setup used to determine the life table parameters of *B. argentifolii* on hibiscus leaf.

cage with an aspirator and moved to a new capsule cage on a fresh leaf every 24 h. Eggs were counted daily using a 14× hand lens and were coded with a number and date using India ink on the nearby leaf surface. Daily monitoring continued until all viable eggs had hatched and all nymphs had pupated. Following pupation, leaf sections bearing pupae were excised and placed individually in glass vials (2 × 4 cm), themselves placed on moist paper towels in a plastic tray until adult emergence. Emerged adults were counted and sexed as described by Gill (1993). The process was initiated with 40 male-female pairs, but only 25 pairs were included in the final data analysis, the remaining pairs having been either lost, physically damaged, or infertile. A life table was constructed using sex ratio, survivorship, and age-specific fecundity of adults and survivorship and developmental of all immature stages to calculate intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_o), mean generation time (T), and doubling time (DT) (Birch 1948), calculated with a computer program by Hulting et al. (1990).

Data Analysis

Data for oviposition and feeding preference of *B. argentifolii* adults on the two hibiscus cultivars were subjected for analysis of variance, and the means were separated using the least significant difference test (LSD) at $P = 0.05$ (SAS Institute 1995).

Results

Adult Feeding and Oviposition Preference

Choice-Test. When given a choice, significantly more *B. argentifolii* adult females were found feeding on 'Pink Versicolor' than on 'Brilliant Red' ($F = 6.08$, df = 1, 23; $P = 0.0175$) (Table 1). Oviposition was also significantly greater on 'Pink Versicolor' than 'Brilliant Red' ($F = 4.42$; df = 1, 23; $P = 0.041$). However, adult fecundity did not differ significantly on the 2 cultivars ($F = 0.25$; df = 1, 23; $P = 0.616$).

No-Choice Test. Significantly more adults were again observed on 'Pink Versicolor' ($F = 6.93$; df = 1, 23; $P = 0.017$, Table 1). However, number of eggs on leaves, and number of eggs deposited per adult female were not significantly different between the two cultivars ($F = 0.89$; df = 1, 23; $P > 0.885$ for eggs/cm^2 leaf area, and $F = 0.01$; df = 1, 23; $P = 0.961$ for eggs/adult female).

Immature Development and Survivorship

B. argentifolii developed almost 2 d faster on 'Pink Versicolor' (22.3 d) than on 'Brilliant Red' (24.1 d) ($F = 23.59$; df = 1, 1728; $P = 0.0001$) with egg and first instar contributing to the difference (Table 2). It was interesting that the most significant differences occurred in length of the egg stage, 6.3 d on 'Pink Versicolor', and 6.7 d on 'Brilliant Red' ($F = 43.97$; df = 1, 318; $P = 0.0001$). Among nymphal stages, developmental times were not significantly different between the two cultivars except for third instar ($F = 11.55$; df = 1, 281; $P = 0.0008$) where the difference was equal (0.4 d) to that observed in egg development. Adult emergence occurred from 19-32 d after oviposition on 'Pink Versicolor' and 19-35 d on 'Brilliant Red'. Estimates for survivorship of immatures in this test were 91.45% on 'Pink Versicolor' compared to 89.79% on 'Brilliant Red', with no significant difference ($F = 2.21$; df = 1, 7; $P = 0.07$). Age specific survivorship on 'Pink Versicolor' and 'Brilliant Red' was not significantly different ($P > 0.05$), and therefore values for the two cultivars were combined (Fig. 2). All eggs

TABLE 1. OVIPOSITION AND FEEDING PREFERENCE OF *B. ARGENTIFOLII* ON 2 HIBISCUS CULTIVARS: ADULT FEEDING AND OVIPOSITION.

	Mean ± SE			
	'Pink Versicolor'	'Brilliant Red'	*F*	*P*
	Two-cultivar Choice Test			
Adults/cm^2	1.67 ± 0.23a	0.94 ± 0.18b	6.08	0.017
Eggs/cm^2	1.45 ± 0.31a	0.72 ± 0.16b	4.42	0.041
Eggs/adult	9.17 ± 1.03a	8.36 ± 1.24a	0.25	0.616
	No-Choice Test			
Adults/cm^2	1.67 ± 0.15a	1.18 ± 0.11b	6.93	0.011
Eggs/cm^2	1.01 ± 0.21a	0.97 ± 0.16a	0.89	0.885
Eggs/adult	9.75 ± 0.31a	9.59 ± 2.34a	0.01	0.961

Means in the same row followed by the same letter do not differ significantly ($P > 0.05$, LSD [SAS Institute 1995]).

hatched successfully; survivorship through successive nymphal stadia was estimated at 96.9%, 92.2%, 88.8%, 84.1%, with 83.4% of the cohort emerging as adults.

Life Table

The estimate for adult longevity was 9.27 ± 0.13 d (range: 4-26 d) (Fig. 3) with 50% adult females surviving for 9-10 d, and >10% for >20 d. Preovipositional period was 0.25 ± 0.01 d (range: 0-1 d). Oviposition varied greatly, and average eggs per female were noticeably fewer than the previous test.

TABLE 2. DEVELOPMENT OF IMMATURE STAGES OF *B. ARGENTIFOLII* ON TWO HIBISCUS CULTIVARS IN THE LABORATORY (26.7°C, RH 55%, 14:10 [L:D] H)

	'Pink Versicolor'		'Brilliant Red'			
Stage	n	Days ± SE	n	Days ± SE	*F*	*P*
Egg	160	6.3 ± 0.6b	160	6.7 ± 0.5a	43.97	0.0001
First instar	157	4.2 ± 0.7a	153	4.3 ± 1.1a	1.64	0.2015
Second instar	147	2.3 ± 0.5a	148	2.4 ± 1.1a	1.66	0.1982
Third instar	145	2.7 ± 0.9b	139	3.1 ± 1.1a	11.55	0.0008
Fourth instar	138	2.6 ± 1.0a	131	2.9 ± 2.4a	1.64	0.2016
Pupa	136	3.8 ± 0.8a	131	4.3 ± 2.9a	3.34	0.0686
Total		22.3 ± 1.6b		24.1 ± 2.2a	23.59	0.0001

Means in the same row followed by the same letters do not differ significantly ($P > 0.05$, LSD [SAS Institute 1995]).

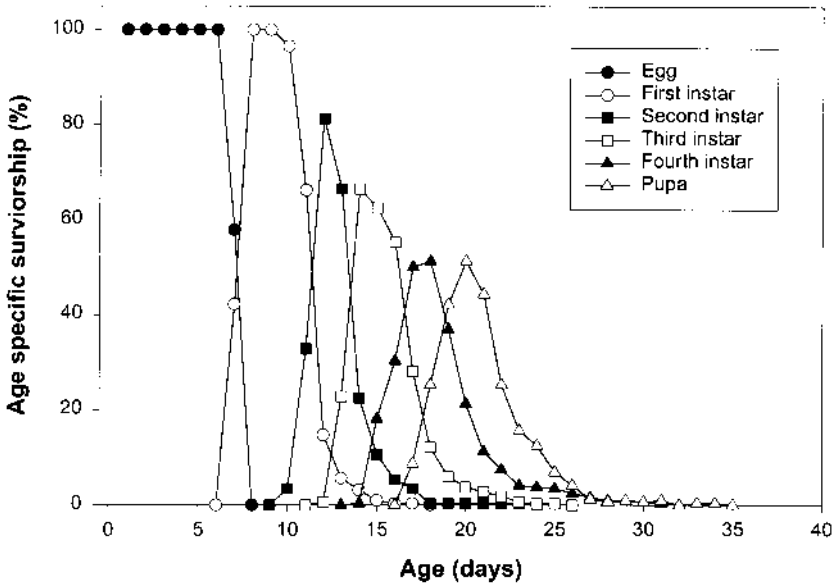

Fig. 2. Age-specific survivorship of *Bemisia argentifolii* immatures on hibiscus ('Pink Versicolor' and 'Brilliant Red') in the laboratory.

Life table parameters for *B. argentifolii* on 'Pink Versicolor' were as follows (Table 3): sex ratio (female: male) 1:0.923, or 52% female (n = 1780), intrinsic rate of increase (r = 0.105) and net reproductive rate (R_o = 17.0). These latter two were lowest of any published values (Tsai and Wang 1996, van Giessen et al. 1995). On the other hand, generation time (T = 27.0 d) and doubling time (DT = 6.6 d) were higher than previously published values (Tsai and Wang 1996).

DISCUSSION

Bemisia argentifolii showed some preference in choice and even no-choice tests for oviposition and feeding on 'Pink Versicolor' compared to 'Brilliant Red', possibly due to the thicker and waxier leaf cuticle, and darker green leaf color typical of latter cultivar. If these characteristics were not tightly linked to flower color they might be transferred to cultivars with the desired light flower shades, thereby possibly reducing whitefly population growth rate.

Compared with other life table parameters of *B. argentifolii* reported from other host plants (Tsai and Wang 1996), *B. argentifolii* also had lower reproductive rate and finite rate of increase, longer generation times, and doubling time. Longevity of *B. argentifolii* on hibiscus (9.3 d) was <50% that on eggplant (24.0 d), and tomato (20.6 d), and also less than on sweet potato (16.6 d), garden bean (13.4 d), or even cucumber (9.9 d).

Small differences in r-values can make remarkable differences in expected population growth over time. For example, r-values on tomato reported by Tsai and Wang (1996) and van Giessen et al. (1995) were 0.153 and 0.122, respectively. Given a stable age distribution, the estimated whitefly population with r = 0.153 from a single female would reach 3873 in a period of 2 generations (54 d), while with r = 0.122, number of whiteflies would only be 726, a 5.3-fold difference.

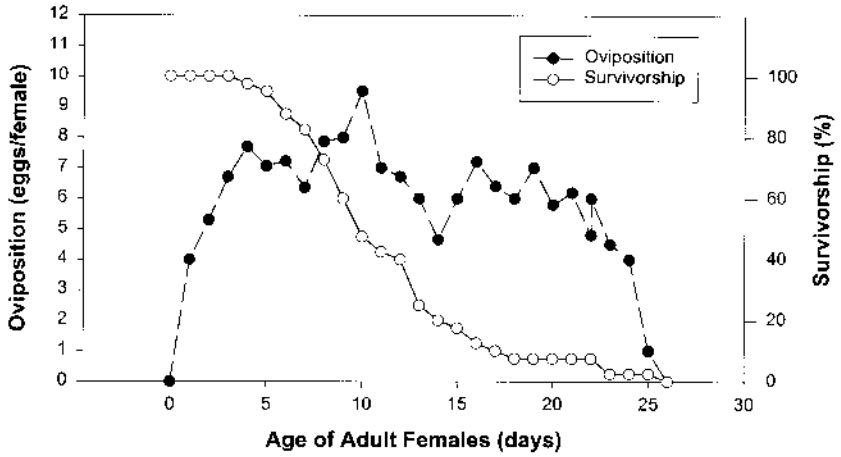

Fig. 3. Survivorship (%) and oviposition of *Bemisia argentifolii* adult females on hibiscus ('Pink Versicolor') in the laboratory.

Performance of *B. argentifolii* on 'Brilliant Red' as indicated by several life history parameters was not as good as that on 'Pink Versicolor'. These parameters on 'Pink Versicolor' were approximately 11% greater than those on 'Brilliant Red', including: (1) oviposition rate per female (9.2 verses 8.4), developmental rate (24 d verses 22 d), and (3) survivorship (91.5% verses 89.8%). Therefore, the *r*-value of *B. argentifolii* on 'Brilliant Red' was simulated based on a series of $l_x m_x$ from the whitefly data on 'Pink Versicolor' with a factor of 11% reduction. To compare the population growths of *B. argentifolii* on the 2 cultivars over time, the number of *B. argentifolii* at time t (N_t) could be calculated as $N_t = N_o e^{rt}$, where N_o is the initial number of whiteflies, r is the intrinsic rate of increase, and t is the time (d). With a logical and biologically sound assumption of r = 0.090 for *B. argentifolii* on 'Brilliant Red', the whitefly population growth on 'Pink Versicolor' from a single female would be 2.25 folds as many as that on 'Brilliant Red' (290 verses 129) in 54 d, or in ≈2 generations.

Given these life history parameters whitefly populations should build up relatively slowly on hibiscus compared to other hosts, and therefore should be easier to manage to whatever population level. Unfortunately, the level of control required in the ornamental market is extremely high. Nevertheless, there could be opportunities early in the crop cycle to utilize biological control and other non-chemical management tactics, given the relatively poor host quality of hibiscus for *B. argentifolii*. In addition, host quality might be further decreased by incorporating foliage characteristics typical of cultivars such as 'Brilliant Red'.

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TABLE 3. COMPARISON OF LIFE TABLE PARAMETERS OF *B. ARGENTIFOLII* ON SEVERAL HOST PLANTS.

Host plants	Intrinsic rate of increase, r	Net reproductive rate, R_o	Generation time, T	Doubling time, DT	Reference
Hibiscus	0.105	17.0	27.0	6.6	This study
Eggplant	0.192	128.2	25.6	3.6	Tsai & Wang 1996
Tomato	0.153	64.3	27.2	4.5	" " " " " " " " " " " "
Sweet potato	0.138	34.6	26.3	5.1	" " " " " " " " " " " "
Cucumber	0.131	19.8	23.2	5.4	" " " " " " " " " " " "
Garden bean	0.120	24.7	27.0	5.8	" " " " " " " " " " " "
Tomato	0.122	—	—	—	Van Giessen et al.
Collard	0.138	—	—	—	1995
Eggplant	0.145	—	—	—	" " " " " " " " " " " "

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EFFECTS OF OIL AND OIL-SURFACTANT COMBINATIONS ON SILVERLEAF WHITEFLY NYMPHS (HOMOPTERA: ALEYROIDIDAE) ON COLLARDS

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ABSTRACT

Collards, *Brassica oleracea* L., treated with SunSpray Ultra-Fine® oil with and without silicon-based oil surfactants were evaluated for effects on nymphs of *Bemisia argentifolii* Bellows & Perring. Synchronously developing populations of eggs, small nymphs and pupae on collards were sprayed to run-off. The rate of egg hatch was not affected by oil treatments. Treated nymphs, pupae, and crawlers emerging from treated eggs, were affected by all oil treatments. Of those nymphs that were affected by the oil, approximately 50% to 75% were killed outright. Over 90% of those that remained alive developed abnormally, remaining small and failing to molt into the next stage. Adults failed to emerge from approximately 94% to 99% of treated pupae. Oil surfactants did not significantly alter whitefly mortality when used with paraffinic oil.

Key Words: insecta, *Bemisia argentifolii*, whitefly, surfactant, oil

RESUMEN

Se evaluó *Brassica oleracea* L. tratada con el aceite SunSpray Ultra-Fine® con y sin un surfactante de aceite en una base de silicón para ver los efectos en ninfas de *Bemisia argentifolii* Bellows & Perring. Poblaciones en desarrollo y sincronizadas de huevos, pequeñas ninfas y pupas en *Brassica oleracea* se rociaron al punto de que chorreaban. El tratamiento de aceite no afectó la eclosión de los huevos. Las ninfas, pupas, y larvas emergiendo de los huevos fueron afectadas por todos los tratamientos de aceite. De las ninfas que fueron afectadas por el aceite, aproximadamente del 50% al 75% murieron al momento. Más del 90% de aquellas que quedaron vivas se desarrollaron anormalmente, manteniéndose pequeñas y no haciendo la muda a la próxima etapa. Adultos no emergieron aproximadamente del 94% al 99% de las pupas tratadas. La mortalidad de las moscas blancas no fue afectada significativamente cuando se usaron en combinación surfactantes de aceite con aceite parafínico.

Whiteflies have been linked to tomato irregular ripening (Schuster et al. 1990) and squash silver leaf (Schuster et al. 1991). Oils have been shown to be toxic and repel-

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lent to adult whiteflies (Liu & Stansly 1995a, Butler & Henneberry 1991a, Butler et al. 1989, Puri et al. 1994), to deter oviposition (Liu & Stansly 1995a, b), to reduce the numbers of nymphs that develop from eggs laid after treatment (Butler & Henneberry 1991a), and to kill treated nymphs (Butler et al. 1993, Puri et al. 1994). Oil, as a control of whiteflies, has the advantages of low toxicity to animals and no restrictions on when sprays can be applied. The disadvantages include the need for repeated treatments (Butler & Henneberry 1991a), crucial need for good coverage (Liu & Stansly 1995b) and potential phytotoxicity (Butler & Henneberry 1991b). Water miscible surfactants are widely used to increase the coverage of insecticidal sprays or to kill insects outright (Chandler 1994). Chemicals that have been added to oil in an attempt to increase repellency, but that had no effect, are trans-cinnamaldehyde, cineole, and citronello (Butler et al. 1989). Oil surfactants are siloxanes that can increase oil spread. We studied paraffinic oil and oil/surfactant combinations for their effects on whiteflies. SunSpray Ultra-Fine® oil was used at 1% as this concentration was previously found to control whiteflies (Liu & Stansly 1995a).

Materials and Methods

The colony of *Bemisia argentifolii* was maintained on collards and tomatoes. To obtain immature whiteflies, 4-wk-old greenhouse-grown collard plants grown in a whitefly-free screened cage were trimmed to three fully expanded leaves and were transferred to the whitefly colony for 48 h. Adults were then aspirated from the plants, and the plants were placed in a separate cage. The synchronously-developing, uniformly-aged whitefly populations were then held until they developed to the appropriate stage. There were approximately 100 to 500 developing immature whiteflies on all of the plants used.

The plants were treated with water (control), 1% SunSpray Ultra-fine paraffinic oil, and 1% SunSpray emulsions with the following silicone-based oil surfactants as 5% of the oil: Silwet 560, Tegoprene 3130, and Tegoprene 6814. The plants were sprayed to run-off with 1.5-l pressurized sprayers. All experiments were done in screened plexiglass cages in a temperature controlled room at 30°C, 50% RH, and 12:12 L:D photoperiod.

Egg Mortality and Attachment of Crawlers

Immediately after the adult whiteflies were aspirated from the plants, the plants were sprayed. There were six replicates (plants) for each of the four treatments. Eight days after treatment, the unhatched eggs and newly emerged nymphs were counted and the percent hatch calculated. The experiment was repeated and the eggs were allowed to develop for 10 d after treatment. Live nymphs, dead attached nymphs and dead nymphs that emerged and did not attach were counted. The percent attachment of newly emerged nymphs and the percentage of nymphs that died after emergence were calculated to determine if attachment affected mortality after hatching.

Early Stage Nymphal Mortality

Eight days after infestation, when first instar nymphs had emerged and attached to the leaf, the plants were sprayed as before. There were 13 replicates (plants) per treatment. Ten days after treatment, the numbers of dead nymphs, large, normally developing nymphs, and small, abnormally developing nymphs were counted and the percentage of each was calculated.

Late Stage Pupal Mortality

Fourteen days after infestation, when most nymphs were in the red-eye stage, the plants were sprayed as before. There were six replicates (plants). Seven days after treatment, when most of the pupae had emerged from control plants, the numbers of empty pupal cases and pupae that failed to emerge were counted and the percent of emergence was calculated.

Arcsin-transformed data were analyzed by ANOVA and means were separated by Student-Newman-Keuls multiple-range test (Gabriel 1964).

RESULTS

Over 90% of the eggs hatched and nymphs were able to emerge, regardless of treatment and no significant differences were detected (Table 1). However, once the nymphs had emerged, they were adversely affected by the oil treatments. Crawlers that hatched from treated eggs attached at a low rate, often dying on empty egg shells. The nymphs that did attach, attached only partially with the posterior abdomen in the air, or died shortly after attaching to the leaf. Mortality of nymphs emerging from eggs that had been treated approached 100%. The differences in attachment or mortality were not affected by the addition of silicon surfactants to the oil.

The percentage of early stage nymphs that developed normally ranged from 1% to 4% for oil treatments compared to 98% for the control (Table 2). Of those remaining, 50% to 76% of oil-treated nymphs were killed outright, and 23% to 44% developed abnormally. Applications of SunSpray oil combined with Silwet 560 had the highest levels of abnormally developing nymphs.

Over 90% of treated pupae failed to complete development to adult emergence (Table 3). The oil appeared to interfere with the ability to emerge into adults. There were no differences among any of the oil and oil-oil surfactant mixtures. Of the pupae that did not complete development at seven days after treatment, some were still alive.

DISCUSSION

Oil is important in the control of whiteflies. It does not prevent neonates from eclosion, but does prevent attachment of first stage nymphs to leaves, and kills those that do attach. Butler et al. (1988) also studied the effect of oil on treated eggs and inter-

TABLE 1. PERCENT EGGS HATCHED, NYMPHS ATTACHED, AND NYMPHS DEAD AFTER EMERGENCE.

Treatment	% Eggs hatched	% Nymphs attached	% Nymphs dead after emergence
Control	95.0 a	—	0.5 a
1% SunSpray Oil®	92.9 a	10.0 a	98.5 b
Silwet 560	93.1 a	25.6 a	99.6 b
Tegoprene 3130	94.0 a	7.8 a	99.6 b
Tegoprene 6814	93.1 a	14.6 a	99.5 b

Arcsin-transformed data were analyzed by ANOVA and the means were separated by Student-Newman-Keuls multiple range test. Means followed by the same letter are not significant ($P = 0.05$).

TABLE 2. PERCENT NORMAL AND ABNORMAL NYMPHAL DEVELOPMENT AND MORTALITY.

Treatment	% Normal	% Abnormal	% Dead
Control	97.9 a	0.1 a	2.0 a
1% SunSpray	4.4 b	29.3 b	66.3 bc
Silwet 560	2.3 b	44.5 c	53.1 b
Tegoprene 3130	1.0 b	23.2 b	75.8 c
Tegoprene 6814	2.0 b	26.6 b	71.4 c

Arcsin-transformed data were analyzed by ANOVA and the means were separated by Student-Newman-Keuls multiple range test. Means followed by the same letter are not significant ($P = 0.05$).

TABLE 3. PERCENT ADULT EMERGENCE.

Treatment	% Emergence
Control	87.7 a
SunSpray	6.0 b
Silwet 560	2.4 b
Tegoprene 3130	1.9 b
Tegoprene 6814	0.7 b

Arcsin-transformed data were analyzed by ANOVA and the means were separated by Student-Newman-Keuls multiple range test. Means followed by the same letter are not significant ($P = 0.05$).

preted a reduction in the numbers of nymphs and pupae as a reduction in egg hatch. Lack of egg mortality from oil treatments was consistent with that observed by Larew and Locke (1990) with SunSpray-treated *T. vaporariorum* eggs. They observed that larvae died in the process of emergence, we noted that death occurred in the emergence process, inability to attach to treated surfaces, or shortly after attachment.

Oil is most effective when applied on eggs for control of first stage nymphs. When first stage nymphs are treated, they are prevented from developing normally by oil treatments with or without the addition of oil surfactants. Some of the nymphs do not die and are not able to molt and grow normally. They appear very rounded, as if they need to, but are not able to molt. This has implications for development of symptoms of tomato irregular ripening, if nymphs are able to continue to live on the plant for long time intervals. Costa et al. (1993) associated whitefly nymphs with the induction of symptoms of squash silverleaf. Oil treatments also prevent the emergence of adults from treated pupae. Again, of the ones that do not emerge, not all are killed outright. Control with paraffinic oil is not affected by the addition of oil surfactants except to impact whether or not the treated nymphs continue to live in an abnormal state or are killed by the oil.

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A NEW SPECIES OF *MELANOPLUS* (ORTHOPTERA: ACRIDIDAE) FROM AN ISOLATED UPLAND IN PENINSULAR FLORIDA

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ABSTRACT

A new species of flightless acridid grasshopper, *Melanoplus withlacoocheensis* n. sp., is described from an isolated upland, the Southern Brooksville Ridge, in west central peninsular Florida. It occurs in open sandhill habitat. It appears to be closely related to *Melanoplus rotundipennis* Scudder, which is widespread in sandhill and Florida scrub habitats in central and northern peninsular Florida; the species are distinguished by details of the male cerci and genitalia. The new species is one of several species of flightless *Melanoplus* in Florida whose geographic ranges are small and biogeographically revealing. The new species is the first clear biological indicator of the long-term isolation of the Southern Brooksville Ridge from other uplands, including the Northern Brooksville Ridge.

Key Words: flightless grasshoppers, biological islands, speciation

RESUMEN

Una nueva especie de saltamonte que no vuela de la familia Acrididae es descrita en esta publicación. Este saltamonte no volador (*Melanoplus withlacoocheenis* n. sp.) fue encontrado en una zona aislada de tierras relativamente altas llamada "Southern Brooksville Ridge" en el interior centro occidental de la península de Florida. Esta especie se encuentra en un hábitat de dunas arenosas. *Melanoplus withlacoocheensis* está cercanamente relacionada a la especie *Melanoplus rotundipennis* Scudder, la cual está ampliamente distribuida en los hábitats de dunas arenosas y de arbustos del centro y norte de Florida. Estas dos especies se pueden distinguir por estructuras de los genitales y los cercos de los machos. *Melanoplus withlacoocheensis* es una de varias especies que no vuelan de este género, las cuales se encuentran distribuidas en rangos geográficos pequeños en Florida, biogeográficamente significativos. Esta nueva especie constituye el primer obvio indicador de un largo aislamiento del área "Southern Brooksville Ridge" de otras tierras altas de Florida, que incluyen el área llamada "Northern Brooksville Ridge".

With the increased interest in the taxonomy and distribution of the insects of North America, biogeographers have identified peninsular Florida as an area of endemism (Allen 1990). Florida is believed to have emerged out of the sea 25 million years ago in the Miocene period (Ashton and Ashton 1985). Since then the sea level has risen and fallen, changing the size and shape of Florida. Locations such as the Welaka area were submerged as much as 90 feet below the water (Friauf 1953). During the largest of these floods, or high water levels, Florida was covered by water, leaving only

a crescent-shaped part of the peninsula in the northwest. The rest of the state was represented by islands of the higher ridges (Hubbel 1984).

The Brooksville Ridge was one of the elevated ridges during this time. The Brooksville Ridge is an area of raised and scattered habitat that spans Hernando and Citrus counties. The Brooksville Ridge is around 25 km wide and 80 km long. Sandhill (high pine) habitat constitutes the largest plant community of the ridge. Sandhill habitat occurs on rolling land with rapid water movement through the soil. It is easily identified by the presence of longleaf pine, *Pinus palustrus*, and turkey oak, *Quercus laevis*. The first sandhill habitats in Florida appeared around 20 million years ago (Myers 1990).

Shaping of the land by alternate flooding and draining affected Florida's flora and fauna. The physical and biotic barriers associated with habitat islands affected the establishment of plants, animals, and insects through isolation (Myers 1990). The isolation of these ridges is still in effect today even after the water has subsided. Ridges are easily considered islands to small flightless grasshoppers if they are bordered by even a small amount of water, unfavorable plant habitat, or civilization. The Southern Brooksville ridge presently is isolated by physiographic features unsuitable for grasshopper survival. It is possible to see that over time these grasshoppers could diverge from their closest relatives.

Melanoplus withlacoocheensis Squitier & Deyrup, **NEW SPECIES**

HOLOTYPE MALE: Body length from front of head to tip of abdomen 15.5 mm. Head width from outer edge of the eyes 3.2 mm. Antennae 5.6 mm long. Minimum distance between eyes on vertex nearly 2 times the maximum width of second antennal segment (scape). Frontal costa diverges gradually with surface shallowly depressed above and below median ocellus. Costa fades out before clypeal suture. Pronotum measures 3.4 mm in length, with three sulci present on pronotum. First two sulci vary in length but not crossing median carina; all three sulci cut lateral carinae. Third sulcus traverses the median carina. Oval tegmina 1.2 times as long as wide. Tegmina extending to first ¼ of the third tergite of abdomen. Prosternal spine rounded. Hind femora 3.5 times as long as wide. Cerci (Fig. 1a) nearly rectangular when viewed from side, with mild constriction near middle, and distal portion rounded. Ventral portion of distal end tapers into a point; upper end expanding into swollen spherical bulb. Cercus length 1.2 mm. Cercus width, viewed from the side, 0.4 mm at depression and 0.5 mm at distal end. Thickness of bulbous portion of cerci 0.5 mm. Furcula reduced to two small rounded lobes. Supranal plate triangular, nearly as long as it is wide at proximal end, extending to rounded point, and with distal edges slightly upcurved. Median groove on supranal plate distinct but short. Pallium erect and measuring 1.2 mm in length. Basal part of penis (Fig. 1b) split from the rear, forming two flaps running ½ the length of the penis; once the flaps join, remainder of penis rounded in cross section. Length of penis 2.5 mm. Orientation of penis from base is 90-degree turn towards front of body followed by 45-degree gradual curve up and away from body. Distal end of penis hollow with a spade culminating in spade-shaped point. Overall body color mottled grayish brown; abdomen and ventral surfaces cream colored. First four tergites of abdomen with black spot on each side and forming line when abdomen is constricted. Black stripe on sides of pronotum extending into pleurites; light cream beneath. Narrow white stripe located on pleurites of thorax beneath wings. Outer surface of femur dark, becoming lighter in color toward lower edge. Inside of femora completely cream colored. Two faint bands on dorsal edges of each femur. Tibiae purple with two columns of black spines.

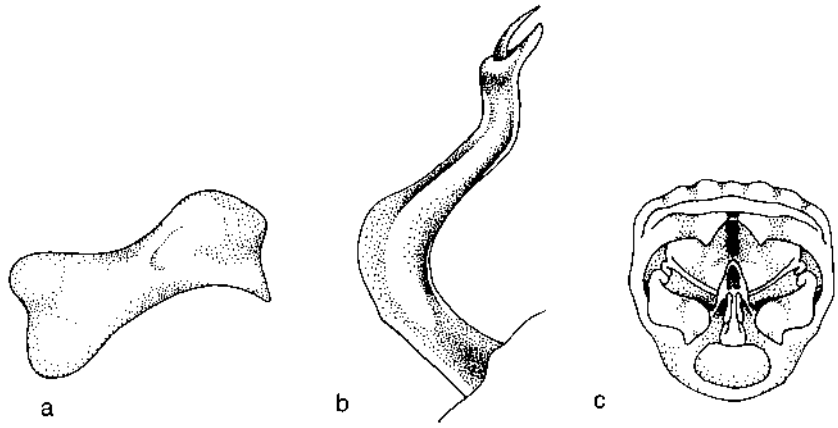

Fig. 1. *Melanoplus withlacoocheensis* n. sp.; a: male left cercus, lateral external view; b: male internal genitalia, lateral view; c: tip of male abdomen, dorsal view.

TYPE LOCALITY: Florida: Hernando Co., State road 50, 5 miles west of Brooksville, collected 15-XI-97. J. M. Squitier.

ALLOTYPE FEMALE: The length of the body from front of head to tip of abdomen is 20.5 mm. The width of the head from the outer edge of the eyes is 3.6 mm. The antennae are 6.8 mm long. The minimum distance between the eyes on the vertex is 2.25 times the maximum width of the second antennal segment (scape). The frontal costa diverges gradually with the medial ocellus evaginated. The costa is as in the male. The sulci are as in the male. The tegmina are 1.8 times as long as wide. The tegmina extends to the first ¼ of the third tergite of the abdomen. The prosternal spine is rounded. The hind femora are 3.9 times as long as wide. The cerci are reduced to small broad triangular shaped processes. There are two indentations on the first abdominal tergite under the wings. Color is the same as the male.

LOCALITY: same as holotype male.

ETYMOLOGY: The specific epithet is derived from the Withlacoochee State Forest, which contains a large population of the species. Suggested common name: Withlacoochee Grasshopper.

DIAGNOSIS: With the unaided eye, males of this species can be separated from all other southeastern *Melanoplus* except for *M. rotundipennis* (Fig. 2) by the greatly enlarged, conical, hood-like pallium. With only moderate scrutiny (a 10× hand lens is adequate) it is easy to see the bulbous apex and ventral tooth of the male cerci in *M. withlacoocheensis*.

The cerci of *M. rotundipennis* (Fig. 2a) are expanded, but not inflated at the tip, and may have an apical ventral angle, but not a ventral tooth-like projection. The cerci also are only about one-half as thick. The penis of *M. rotundipennis* is somewhat variable through its range (Fig. 3), but is never strongly sinuate; the penis of *M. withlacoocheensis* is also larger, generally about 1.5 times as long, as that of *M. rotundipennis* (Table 1). The penis of *M. withlacoocheensis* has a distinct S-shaped curve and is round in cross section, whereas in *M. rotundipennis* there is one gradual curve along the entire structure and it is oval in cross section. We have not found reliable characters for separating females of *M. withlacoocheensis* and *M. rotundipennis*. A discussion of the relationship between these two species appears below.

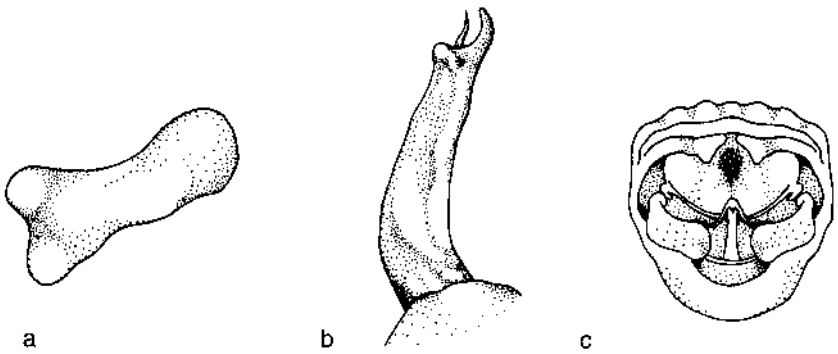

Fig. 2. *Melanoplus rotundipennis*; a: male left cercus, lateral external view; b: male internal genitalia, lateral view; c: tip of male abdomen, dorsal view.

PARATYPES: Two males, 1 female, same locality, date, collector as holotype; 3 males, 1 female, Florida, Hernando Co., 11.2 km south of junction U.S. Route 19 and State Road 50, along road, 15-IX-1997, J. M. Squitier; 4 males, 4 females, Florida, Citrus Co., Withlacoochee State Forest, 1 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 stricta*, scattered mature *Pinus palustris*, *Quercus laevis*, 3-XI-1991, M. and N. Deyrup; 1 male, 2 females, same forest road and habitat as previous record, 3.2 km south on forest road, 14-XI-91, M. Deyrup; 4 males: same forest road and collection data as previous record, 14.4 km south on forest road; 1 male same data as previous record, but 4.8 km south on forest road; 1 male, same data as previous record, but 12.8 km south on forest road; 1 male, 1 female: 9.6 km west of Brooksville, on State Road 50, open sandhill habitat on south side of road, 26-VIII-92, M. Deyrup and Z. Prusak.

DEPOSITION OF TYPE MATERIAL: Holotype male, allotype female, 4 males: Florida State Collection of Arthropods, Division of Plant Industry, Gainesville, Florida; 3 males, 2 females: U.S. National Museum of Natural History, Washington, D.C.; 3 males, 2 females: Academy of Natural Sciences, Philadelphia, Pennsylvania; 3 males, 2 females: University of Michigan Museum of Zoology, Ann Arbor, Michigan; 3 males, 1 female: Archbold Biological Station arthropod collection, Lake Placid, Florida; 2 males, 1 female: collection of Department of Entomology and Nematology, University of Florida, Gainesville, Florida.

HABITAT: All specimens were collected in sandhill (high pine) habitat; for a detailed description of this habitat and its dependence on frequent fires, see Myers (1990). The specimens were found in open areas with a sparse to moderately sparse ground cover of wiregrass, *Aristida stricta* Michx., mixed with other herbaceous plants, such as *Pityopsis graminifolia* (Michx.) Nutt. (the most common species), *Polygonella robusta* (Small) Horton, *Paronychia* sp., *Balduina angustifolia* (Pursh) Robins, and *Chrysopsis scabrella* Torr. and Gray. There were scattered mature trees of *Pinus palustris* Mill., *Quercus laevis* Walt., and *Q. incana* Bartr., and in some collecting sites there were *Quercus geminata* Small, *Quercus chapmanii* Sarg., and *Quercus stellata* Wang. Insects found regularly at the collecting sites and represented by voucher specimens include the Acrididae *Melanoplus puer* (Scudder) and *Eritettix obscurus* (Thomas) and *Achurum carinatum* (F. Walker), the Tettigoniidae *Odontoxiphidium apterum* Morse, the Formicidae *Odontomachus clarus* Roger, *Pogono-*

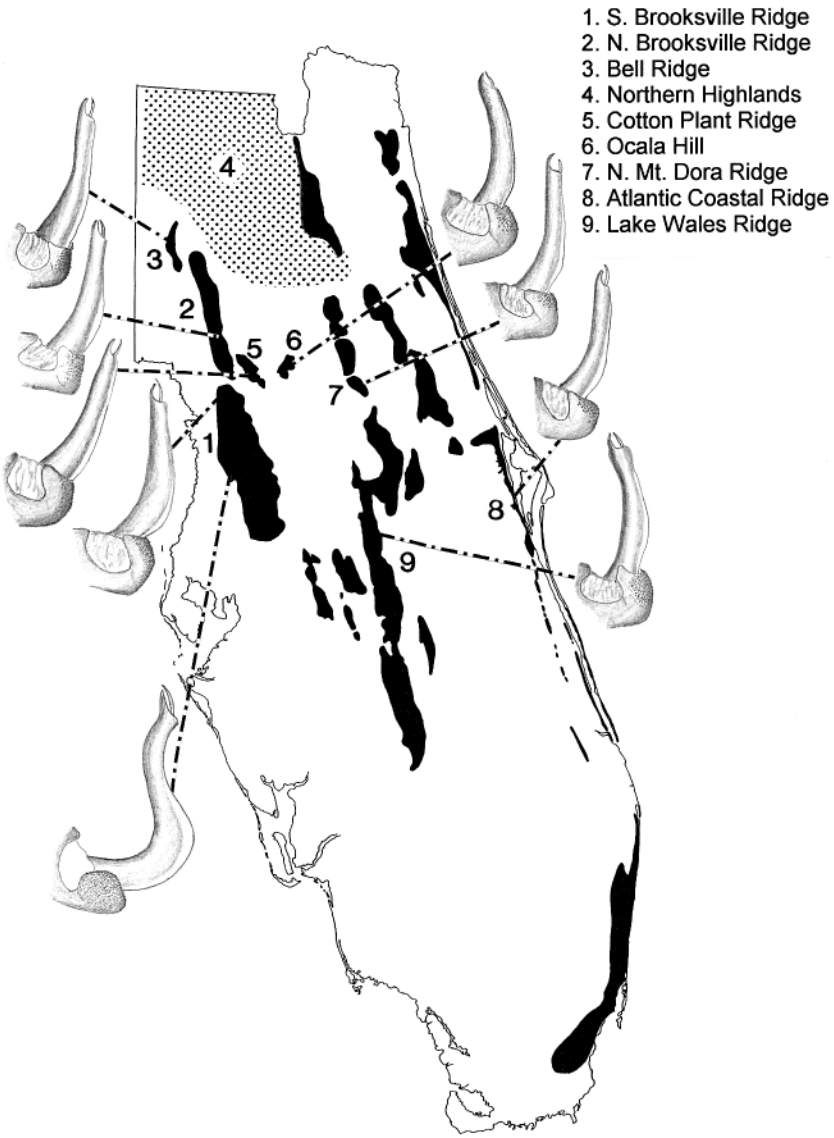

Fig. 3. Sand ridges of peninsular Florida (redrawn from White 1970), showing internal male genitalia of *M. withlacoocheensis* (drawing in lower left) and eight populations of *M. rotundipennis.*

myrmex badius (Latreille), *Pheidole morrisi* Forel, *Monomorium viride* Brown, *Leptothorax texanus* Wheeler, *Trachymyrmex septentrionalis* (McCook), *Dorymyrmex* cf. *elegans* (Trager), *Camponotus socius* Roger, *Paratrechina arenivaga* (Wheeler), and *Formica pallidefulva* Emery. All these associated plants and insects are typical of san-

TABLE 1. SIZE VARIATION (MM) IN SOME SPECIMENS (N = 10) OF *M. WITHLACOOCHEENSIS* AND *M. ROTUNDIPENNIS*.

	MALE		FEMALE	
	M. withlacoocheensis	*M. rotundipennis*	*M. withlacoocheensis*	*M. rotundipennis*
Body length	15.4-16.5	13.0-16.0	20.0-20.5	16.7-21.3
Antenna length	6.3-6.5	5.8-7.0	6.6-7.0	6.2-8.0
Tegmen length	3.1-3.5	3.0-3.7	3.7-4.1	3.6-4.6
Tegmen width	1.6-1.9	1.6-1.9	2.1-2.2	1.7-2.5
Femora length	9.0-9.7	9.4-11.0	11.2-11.8	11.4-13.2
Femora width	2.4-2.6	2.4-2.8	2.9-3.1	3.0-3.3
Cercus length	1.1-1.3	1.1-1.4	0.5-0.6	0.5-0.6
Cercus thickness	0.4-0.5	0.1-0.2	0.2-0.3	0.1-0.2
Cercus min width	0.4-0.5	0.3-0.4	NA[1]	NA[1]
Cercus distal width	0.4-0.6	0.3-0.6	NA[1]	NA[1]
Cercus basal width	0.6-0.7	0.6-0.8	0.4-0.5	0.4-0.5
Adeagus length	1.9-2.7	1.3-1.6	NA[2]	NA[2]

[1]Shape of cercus in female is triangular.
[2]Present in males only.

dhill habitat through peninsular Florida, except for *Odontomachus clarus* and *Dorymyrmex elegans*, which are confined to the Lake Wales and Orlando Ridges in the interior of the state.

Discussion

Relationships of *M. withlacoocheensis*

Flightless *Melanoplus* species are similar to one another in appearance. The best features one has for identification are the cerci and adeagus of the male. The cerci are easily viewed by eye or with a hand lens while the adeagus (penis) must be physically exposed, usually under a dissecting microscope. *Melanoplus withlacoocheensis* is closely related to *M. rotundipennis*. Although the differences between the two species in male cerci and internal genitalia (see Figs. 1 and 2) may seem dramatic by the standards of interspecific divergence seen in many other groups of insects, these are small differences compared with those separating most other flightless *Melanoplus* species. Therefore, it is possible that this population might represent a clinal extreme of *M. rotundipennis*, a species that was already known to show some geographic variation in male cerci and internal genitalia (Hubbell 1932). We therefore collected samples from populations throughout its range in Florida, with special emphasis on populations inhabiting central Florida's upland ridges that might have been isolated by adjacent lowlands, especially those ridges that have produced distinctive, often allopatric, species of flightless *Melanoplus* (Deyrup 1996). The internal genitalia of representatives of these populations of *M. rotundipennis* are shown in Fig. 3. This not only shows that there is no trend toward the highly sinuate penis of *M. withlacoocheensis,* but also that *M. rotundipennis* shows no tendency to give rise to notably divergent populations elsewhere in peninsular Florida. More recently, we have found specimens of typical *M. rotundipennis* at the northern end (Pine-Oak Estates) of the Southern Brooksville Ridge, and the two species occurring sympatrically on the east-central edge of the Southern Brooksville Ridge (1.5 km west of Nobleton) (vouchers in Archbold Biological Station arthropod collection).

Between this pair of species and any other *Melanoplus* there is a great gap in terms of male genitalic morphology. This is not unusual among the flightless *Melanoplus*, a group in which there appears to be rapid divergence of male genitalic morphology, probably driven by sexual selection (Deyrup 1996), combined with a tendency for populations to become isolated on "islands" of habitat (Hubbell 1961). In his "*rotundipennis* group," Hubbell (1932) included one other species, *M. pygmaeus* Davis, chiefly on the basis of the expanded and spatulate tips of the cerci. This reflects the earlier judgement of Davis (1915) that *M. rotundipennis* and *M. pygmaeus* are related, and sensibly removes these two species from the *puer* group. In addition to the distal expansion of the cerci, *M. pygmaeus* and *M. rotundipennis* resemble each other in general size and markings. They are both relatively common inhabitants of sandhill habitat, and always associated with wire grass (*Aristida stricta* and its relatives) and scattered upland oaks. The two species are separated by about 120 km, including the Ochlockonee and Apalachicola River systems. With the addition of *M. withlacoocheensis*, the *rotundipennis* group now includes three recognized species, but with more analysis additional species might be found that fit into this group among southeastern *Melanoplus* associated with high pine habitats.

The description of *M. withlacoocheensis* raises the number of Florida flightless *Melanoplus* species to about 18. In the keys provided by Blatchley (1920) and Hubbell (1932), this species keys to *M. rotundipennis*. It will soon be possible to identify Flor-

ida *Melanoplus* using a new manual of Florida grasshoppers (Capinera et al. 1998), and Daniel Otte is engaged in the major task of revising the North American species of *Melanoplus*. This latter effort, in particular, should reveal the high level of speciation among the short-winged *Melanoplus* spp.

Biogeography of *M. withlacoocheensis*

The Withlacoochee grasshopper appears to be a classical peripheral isolate (as discussed in Mayr 1963) of the *rotundipennis* lineage. We believe that *withlacoocheensis* is an isolate, rather than a relic of a lineage ancestral to *rotundipennis* because the male cerci and internal genitalia are more elaborate in *withlacoocheensis*, in accordance with the normal course of sexual selection. If we hypothesized that *withlacoocheensis* was the less derived species, we would be forced to invoke other phenomena, for which we have no evidence, to explain the more widespread distribution of *rotundipennis* with its less elaborate structures.

The Withlacoochee grasshopper is known only from the Southern Brooksville Ridge, isolated to the north by the Withlacoochee River, which bisects the Brooksville Ridge, to the east by the Tsala Apopka lowlands, to the west by the coastal lowlands, and to the south by the Zephyr Hills Gap (the names of these features are from White 1970). The large sand ridges of the Florida peninsula are likely to maintain islands of xeric habitat in long-term isolation because some of the rain that falls on these ridges percolates out to the sides in the surficial water table to support extensive seeps and strips of swamp forest. Even during dry periods, such as those that prevailed during various times in the Pleistocene (Webb 1990), enough water might be slowly moving out from the edges of the larger ridges to support a mesic habitat that would serve as a barrier to the movement of flightless grasshoppers adapted to xeric habitats. This was recognized and discussed by Hubbell (1932), who made a special effort to visit some of the ridges. He may even have seen *M. withlacoochee*, as he mentions (1984) an undescribed *Melanoplus* from western peninsular Florida (the first definite reference to the species is in Deyrup 1996).

The massive sand deposits that form the Brooksville Ridge and other inland ridges and uplands in Florida were washed down from the north at the end of the Miocene and during the Pliocene, and the higher parts of the Brooksville Ridge are among the oldest and most continuously exposed sites in Florida (Scott 1997). Theoretically, therefore, the Brooksville Ridge has been available for colonization for several million years, and would not have been completely inundated even during the high stand of the sea (32-35 m above present) that left the Wicomico shoreline in the early Pleistocene (Webb 1990). The dry longleaf pine savannahs that are the present habitat of *M. rotundipennis* and *M. withlacoocheensis* also appeared in Florida in the early Pleistocene, so this stock could theoretically have occurred in Florida for roughly 1.5 million years (Webb 1990). This was the peak of the migration of dry savannah species from southwestern North America (Webb 1990), so the *rotundipennis* group may have a western origin, although it is more conservative to consider it an endemic southeastern lineage until there has been more analysis of *Melanoplus* phylogeny. The isolation of the southern Brooksville Ridge by the Withlacoochee River might have been a crucial development for the isolation of the ancestors of the Withlacoochee grasshopper, and the narrowness of its cut through the unconsolidated sands of the Brooksville Ridge suggests that this is not a particularly old river valley. To summarize, *M. withlacoocheensis* is probably a relatively recently derived species (as species go), probably a million years old or less, and the present distribution suggests that it evolved on the Southern Brooksville Ridge, which has become accessible to typical *M. rotundipennis* only recently, perhaps during a dry period of the late Pleistocene glaciations, or per-

haps since humans began to drain the area, and establish open grassy road shoulders leading from one isolated upland to another. This species and other flightless *Melanoplus* would be ideal subjects for combining geological evidence with biochemical techniques to show patterns of speciation.

The Withlacoochee grasshopper is an important indicator species in the effort to preserve the diversity of Florida's upland habitats because it is the first example of a species endemic to the xeric habitats of the Southern Brooksville Ridge. It is unlikely that this is the only species isolated on this ridge, and surveys for other new endemic species are in order. Some plants to examine carefully are members of the genera *Lechea, Paronychia, Chrysopsis, Galactia, Tephrosia*, and *Bulbostylus*. Some insects to check are crickets of the genera *Gryllus, Pictonemobius*, and *Cycloptilum*; beetles of the genera *Selenophorus, Selonodon, Psammodius, Phyllophaga, Serica, Anomala, Mecynotarsus, Pleotomodes,* and *Blapstinus*; flies of the genera *Nemomydas, Townsendia, Phthiria, Glabellula,* and *Asyndetus*; Hymenoptera of the genera *Photomorphus, Tachytes* and *Perdita*. The time of expeditions of discovery in Florida is far from over.

Acknowledgments

The drawings of *Melanoplus withlacoocheensis* and *M. rotundipennis* were made by Keira Dooly. Daniel Otte has been consistently helpful and encouraging in our work with Florida *Melanoplus.* Howard Frank made useful comments on an early draft of the manuscript. The following people helped hunt for the new species and its relatives, including some expeditions when there were no grasshoppers to be found: Lloyd Davis, Paul Skelley, Clay Scherer, Stephen Lenberger, Glen Lenberger, Zachary Prusak, Nancy Deyrup, Stephen Deyrup. We thank the managers of the Withlacoochee State Forest for permission to study grasshoppers in this forest, and we are even more grateful for their efforts to maintain the species-rich sandhill habitat which is the home of this interesting grasshopper. Published as Florida Agricultural Experiment Station Journal Series No. R-06416.

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SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) ATTACK ON GROUND MONITORS AROUND AN APARTMENT COMPLEX IN FIXED PATTERN PLACEMENTS VERSUS CONDUCIVE PLACEMENTS

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One of the newest tools for the management of subterranean termites (*Reticulitermes* spp., *Coptotermes formosanus* Shiraki, and *Heterotermes* spp.) is termite baiting. Limitations to the effective use of baits include difficulty in getting the termites to accept baits and the need for baits to remain in place for an extended period of time to allow slow-acting toxicants to work. One factor that may have importance to bait acceptance is bait placement. Su et al. (1984) found that foraging by subterranean termites is a random process of investigation and suggested that termites do not discriminate among similar food sources within a colony's foraging range. This suggests that toxic baits placed randomly or uniformly within a foraging range of a colony will have equal chance of discovery. Therefore, the best strategy for bait placement would be to employ them in a manner that is most convenient to the pest control operator or acceptable to state regulators. A preset patterned placement of baits around all structures in a similar manner makes baiting procedures easy to follow, location of baits during follow-up inspections simple, and requires little to no background information on termite activity in the area before implementation of the process. However, Delaplane and La Fage (1987, 1989) and Oi et al. (1996) found clear preferences were made by subterranean termites, even when food sources were identical, and argued that termites do not feed randomly. The most important variable for increased preference was relative proximity to termite activity. Thus, baits clustered close to termite activity or areas conducive to termite habitation might increase the probability of termite feeding. The objective of this study was to determine which placement type is the more effective method of using wood monitor stations to locate subterranean termites (both native species, *Reticulitermes spp.,* and Formosan, *Coptotermes formosanus)* around buildings in an urban habitat.

The study site consisted of 11 apartment buildings (Georgetown Apartments, New Orleans, LA), similar in size and construction and located in a known area of Formosan subterranean termite infestation. For each apartment, diagrams were drawn detailing exterior areas that were prone to moisture problems or had readily available termite food, such as exterior water faucets, air conditioners, downspouts, landscaping timbers, mulch beds, wooden fencing, and tree stumps. These areas were considered more likely to harbor populations of subterranean termites nearby (areas conducive to termite infestation). Pine stakes (2.5cm × 5cm × 30cm) purchased from a local hardware store were placed around each of the 11 similar apartment buildings. Approximately 20 stakes were placed in a fixed (uniform) pattern around each structure, and another 20 were put in a conducive placement pattern, for a total of 428 stakes. For the uniform placement, stakes were driven into the ground 3.3m away from the building and 5m away from each other. For conducive placements, stakes were positioned at least 30cm away from the building in conducive condition areas (i.e. near water and food sources where termites were likely to concentrate their ac-

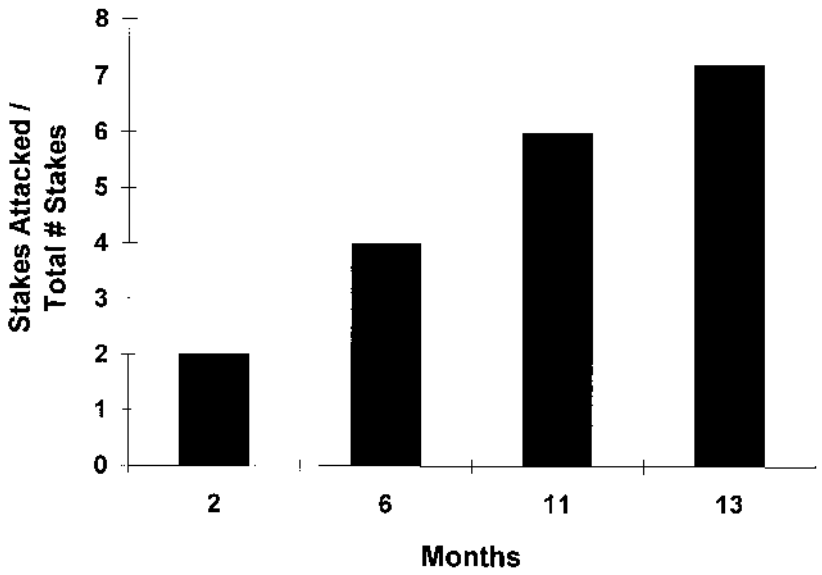

Fig. 1. The percentage of stakes attacked at each inspection period.

tivities, as described above). Each side of the buildings received approximately the same number of conducive and uniformly placed stakes. Stakes were inserted all the way into the ground so as to be inconspicuous and not interfere with normal landscaping activities. Stakes were placed in the ground in July, 1995, and all were inspected for termite activity after two, six, 11, and 13 months. Both Formosan subterranean termites, *Coptotermes formosanus,* and native subterranean termites, *Reticulitermes* spp., occupied this area.

The incidence of stake attack was low throughout the study, with only about 7% of the pine stakes showing signs of attack after 13 months (Figs. 1 and 2). On average, 73% of stakes attacked were found to harbor termites on the next inspection. Of the wooden stakes that were attacked, stakes in conducive placements were attacked twice as often as fixed patterned stakes. The stakes around one building amounted to over one-half (16) of the total number of attacks on all stakes (fixed and conducive) at month 13. Conducive placement stakes were seven times more likely to be attacked at this building compared with stakes in a uniform pattern. We tested for the statistical significance of stake placement and termite attack using generalized linear modeling and analysis of deviance (SAS Institute, Cary, NC) where the indicator variable was stake type and the class was building effect. Wald statistics generated an odds ratio association with stake type. There was no significant difference in stake placement for termite attack.

Our findings suggest that a sound knowledge of termite biology and foraging behavior will increase the success rate in locating termites with ground monitors. We know that termites show preferences when certain amino acids are added to foods (Chen and Henderson, 1996). Thus, both bait placement and bait quality are important variables that can improve bait acceptance.

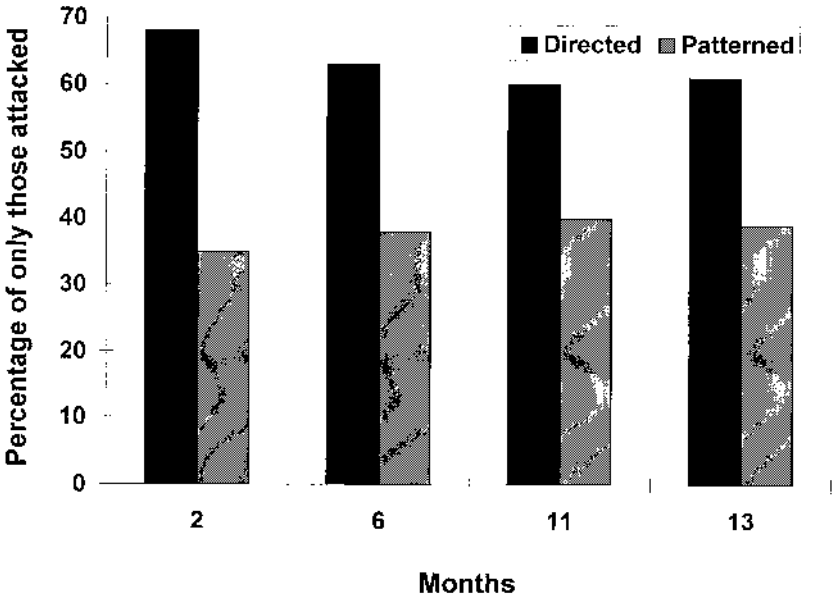

Fig. 2. Of the stakes that were attacked, the percentage that were attacked in directed vs. patterned placements at each inspection period.

Our study was unable to determine how many stakes were contacted by termites but not fed upon. So, although foraging may be nonrandom, the strategy in search for food may not. Oi et al. (1996) stated that search behavior is random in subterranean termites, although no data to support this conclusion are provided. Robson et al. (1995) found search to be a nonrandom process in *Reticulitermes flavipes* (Kollar). Research on search behavior will help our understanding of subterranean termite foraging behavior and its applications toward successful termite control.

SUMMARY

Four hundred and twenty-eight wooden stakes were placed in the ground around eleven similar buildings using conducive and uniform placements to evaluate placement success relative to termite attack. Stakes were monitored for termite attack at two, six, 11, and 13 months. Overall, monitors placed in conducive locations were twice as likely to be attacked by subterranean termites compared with patterned placements; however, the difference was not statistically significant.

Redd Pest Control, Kenner, LA, helped in selecting the 11 buildings. We thank Dr. Brian D. Marx (LSU, Experimental statistics) and Beverly Wiltz (LSU, Dept. Entomology) for technical assistance. Research support for this project was provided by the Louisiana Department of Agriculture and Forestry, which administers funding from pest control operators of Louisiana, and a grant from FMC Corp. Publication for this research was approved by the Louisiana State University Agricultural Center and Louisiana Experiment station as manuscript number 97-17-0232.

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ERRATUM

A Review of the Literature on *Toxoptera citricida* (Homoptera: Aphididae). By J. P. Michaud 81(1): 37-61.

The last sentence on page 38 is in error and should read as follows: The BCA is still absent from the Mediterranean region (Mendel, 1956; Jamoussi 1967), including Turkey (Yumruktepe & Uygun 1994).

BOOK REVIEWS

POTTER, D. A. 1998. Destructive Turfgrass Insects. Biology, Diagnosis, and Control. Ann Arbor Press (a division of Sleeping Bear Press); Chelsea, Michigan, xvi + 344 p. ISBN 1-57504-023-9. Hardback. $65.00.

The size [7" × 10" (17.8 × 25.4 cm)], cover design, and paper quality of this book remind me strongly of Leslie (1994) which I found to be a valuable reference source. The author of this book was able to capitalize on information presented in Leslie (1994) but focus on pests of turfgrass. That was an advantage for this book. Its author has adopted an excellent arrangement of the contents into the following chapters: 1. Managing turfgrass insect pests; 2. Insect biology and identification; 3. Detection and monitoring of insect pests; 4. Insecticides—types and mode of action; 5. Using insecticides safely; 6. Using insecticides effectively; 7. Safeguarding the environment; 8. Root-infesting insect pests; 9. Pests that burrow in stems or damage crowns; 10. Pests that suck juices and discolor leaves and stems; 11. Insects that chew leaves and stems; 12. Biting and stinging pests in the turf environment; 12. Nuisance pests and innocuous invertebrates; 14. Beneficial invertebrates: Predators, parasitoids, and thatch builders; and 15. Managing nuisance wildlife problems in the turfgrass environment. There are also 4 appendices, a glossary of terms, and an index. The book includes 32 plates of color photographs of insects or the damage they cause, which are far more useful than black-and-white although the colors of some are distorted and others are of lesser quality.

The second and subsequent editions of this book deserve to become standard reference works. These should attempt to replace the worst of the color photographs, and correct some of the little errors that should have been caught by peer reviewers. Here are some of them. Page 30 suggests that the name of a species is one word, although zoology adopted a binominal nomenclature in 1758. Pages 33 and 266 misspell Cicindelidae. A canvas sweepnet with D-shaped mouth with the flat side down would be more practical in turf than the net illustrated on p. 47. Page 312 implies that the word "exuviae" has a singular "exuvium" whereas it has no singular form in English any more than does the word "feces" (The Torre Bueno Glossary of Entomology, 1989, New York Entomological Society).

A worse fault arises from the author's lack of experience in the southern USA: he has attempted not entirely satisfactorily to use literature known to him to fill in gaps in his knowledge. His review of mole crickets, which are the most important pests of turfgrass in the USA by virtue of the damage they do in the coastal plains of the South (Golf Course Management 63[5]: 22), is better than any I have seen written by others without experience, but misses some points, including some very important points. He represents their names correctly, barring the author (Perty), see p. 121, of *Neocurtilla hexadactyla*. He states (p. 122) that their eggs are "round, translucent, and whitish" whereas their color is grayish brown and not translucent, and their shape is ovoid. His map (p. 122) shows the tawny mole cricket as occurring in western Arizona, whereas only the southern mole cricket, as his text states, has been found there (Florida Entomol. 71: 90-91). This same map fails to show the presence of the tawny mole cricket in eastern Texas, although the text states that it is there. It also shows the southern mole cricket to occur in unexplained places that appear to be north of Louisiana or Texas. In the Virgin Islands, the short-winged mole cricket is known only from St. Croix (Florida Entomol. 69: 760-761, 79: 468-470), not from the other islands.

He states (p. 129) that the wasp *Larra bicolor*, a biological control agent of *Scapteriscus* mole crickets, was imported into south Florida and does not seem to have had a major effect on mole cricket populations. That statement would have been almost correct, so far as was known in early 1993 (in fact, wasps were released in other parts of

Florida but did not become established), but another biotype of the wasp was released, became established in north Florida, and is spreading (Florida Entomol. 78: 619-623).

He states (p. 129) that the tachinid fly *Ormia depleta* was imported from Brazil and established "throughout most" of Florida but does not seem to have a major effect on mole cricket populations. In fact, the fly is established in 38 contiguous counties of peninsular Florida to 29°N, but neither in the far north nor in the panhandle (Biological Control 6: 368-377) and **does** have a major effect on mole cricket populations (Environ. Entomol. 25: 1415-1420). Unpublished information underscores the major effect of this fly.

He writes (p. 128-129) about the entomopathogenic nematodes *Steinernema scapterisci* and *S. riobravis* as biopesticides. His brief account misses some very important points. The nematode *S. scapterisci* is not very effective against short-winged mole crickets (I do not know of information about the effect of *S. riobravis* on this mole cricket) but is effective against adult southern and tawny mole crickets. In contrast to *S. riobravis*, which is native to Texas and is not a specialist on *Scapteriscus* mole crickets, *S. scapterisci* is a South American specialist on these mole crickets and only it is known to reproduce in them, release infective juveniles into the soil, and establish populations in the field. The only published record for persistence of such *S. scapterisci* populations is 5 years (J. Entomol. Sci. 25: 182-190), but it has been shown to spread by flight of infected mole crickets from places of release (Florida Entomol. 75: 163-165, 76: 75-82), and there is overwhelming unpublished evidence that such populations not only are permanent, but have spread and are spreading to other localities in Florida. Thus, *S. scapterisci* is an inoculative biological control agent when used against *Scapteriscus*, as was stated in Leslie (1994), not merely a biopesticide. This makes *S. scapterisci* a far more useful biological control agent than indicated by this book's author.

The author of this book has, in general, done a fine job in its preparation. He has simply overlooked some of the more recently published research on biological control of mole crickets. As major source for research on mole crickets he has cited only extension publications, including Walker (1985) which is by far the most thorough yet published, but >14 years have passed since its preparation. I hope that future editions of this book will correct the deficiencies and make it the best general reference work available for the entire country, not just the North.

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WOODRUFF, R. W., B. M. BECK, P. E. SKELLEY, C. Y. L. SCHOTMAN, AND M. C. THOMAS. 1998. Checklist and Bibliography of the Insects of Grenada and the Grenadines. Center for Systematic Entomology; Gainesville, Florida. Memoir 2, 286 p. ISBN 1-877743-28-3. Hardback. Price per copy $58 ($29 to members of CSE) plus $2.00 postage and handling to USA addresses ($5.00 abroad), from The Treasurer, Center for Systematic Entomology, P.O. Box 147100, Gainesville, FL 32614-7100.

This is the second volume of the Memoir Series of the Center for Systematic Entomology (CSE). It is the product of a cooperative program between the FAO, Grenada's

Ministry of Agriculture, and the Florida Department of Agriculture and Consumer Services. The part of the product presented here is the result of a review of the literature about insects in Grenada.

The first thing I noticed about this book was its front and back covers which bear fine color photographs taken by the senior author of the flowers of *Delonix regia* and of Concord Falls respectively. Both are sights to be seen in Grenada, which is among the most scenic islands in the Lesser Antilles. The Grenadines are tiny islands extending northward, some of which belong to Grenada and some to St. Vincent.

Most of the pages of the book are a checklist of the scientific names of insect species, each with the citation (author, year and page number) of the original description. These names are arranged alphabetically within families, which in turn are arranged alphabetically within orders, and the orders themselves are arranged alphabetically. This arrangement just about eliminates the need for an index, so the book has only a 3-page index of family names. Against most of the species names is listed the distribution of that species in places other than Grenada, but more especially in the West Indies. Under many of the names of families is given the name of a specialist taxonomist who reviewed the included information for correctness before publication. Under the name of each insect order are a few paragraphs and some literature citations. Some of the family names also are followed by an even briefer text and similar citations. The bibliography occupies 75 pages, and some of the items are annotated. Despite its length, the authors admit that the bibliography is not exhaustive and, for example, does not include all of the references to accompany the author, year, and page of original descriptions of insects.

A 3-page introduction to the book gives a thumbnail history of entomology in Grenada. The checklist is incomplete because, unsurprisingly, some species are not yet recorded. In fact, part of the project in which the senior author was heavily involved was to make new collections of insects from Grenada and work toward their identification. Few publications have yet resulted from work on these collections, but many specimens are now available to specialists.

The most similar checklist of the insect fauna of a West Indian island is **Catalogus Insectorum Jamaicensis** by C. C. Gowdey, published in 3 parts in 1926-1928 by Jamaica's Department of Science and Agriculture. It includes brief new taxonomic studies of small parts of the fauna by three specialists (C. P. Alexander, C. H. Curran, and W. S. Fisher) and lacks a bibliography. In volume 13 of Scientific Survey of Puerto Rico and the Virgin Islands, published by the New York Academy of Sciences in 1970, G. W. Miskimen and R. M. Bond produced a checklist entitled **The Insect Fauna of St. Croix, United States Virgin Islands**. The most celebrated checklists are the heavily annotated and partially illustrated lists written by G. N. Wolcott to the insect fauna of Puerto Rico, first as **Insectae Portoricensis** (1924), and revised as **Insectae Borinquenses** (1936-1941). Those lists metamorphosed into a 748-page book, **The Insects of Puerto Rico**, by the same author, published in parts in 1950-1951 (actual dates) by the Journal of Agriculture of the University of Puerto Rico; this was no longer a checklist, but a work dealing with the natural history of Puerto Rico's insect fauna.

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PHOTOGRAPHS FROM
THE 80TH ANNUAL
MEETING OF THE
FLORIDA
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DAYTONA BEACH

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Fig. 1. With a look of stern approval, outgoing President David Hall presents the gavel to incoming President Joe Funderburk (right).

Fig. 2. President Funderburk presents the Past President's Award to David Hall for outstanding dedication and service as president of FES for 1997.

Fig. 3. Thomas Walker receives a Presidential Recognition Award for his vision, leadership, and devotion to the publication of the Florida Entomologist on the Internet.

Fig. 4. David Williams accepts the FES Achievement Award for Research from President Funderburk.

Fig. 5. The FES Achievement Award for a Collaborative Research Team is presented to Ru Nguyen (center) and Margorie Hoy (right).

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Fig. 6. President Funderburk (left) presents the FES Achievement Award for Industry to Dean Remick.

Fig. 7. John Sivinski receives a Certificate of Appreciation for his service as the Chairman of Local Arrangements Committee.

Fig. 8. Certificates of Appreciation are awarded to Nancy Epsky (not pictured) and Lois Wood for service as Co-Editors of the FES Newsletter.

Fig. 9. Clay Scherer accepts a Certificate of Appreciation for Student Member of the Executive Committee.

Fig. 10. Chairman of the Honors and Awards Committee Gary Leibee (left) presents Edward Knipling with a FES Pioneer Lecture Honoree Award.

Fig. 11. Alfred Baumhover is recognized for his dedicated efforts as a key entomologist on the Screwworm Eradication Team.

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Fig. 12. Juan Villanueva-Jimenez receives a $500 FES scholarship from President Funderburk.

Fig. 13. Dina Richman accepts an award for her student paper presentation.

Fig. 14. Moh Leng Kok-Yokomi (not pictured) and Gary Leibee receive Certificates of Appreciation for serving as FES Special Awards Judges at the 42nd State Science and Engineering Fair of Florida.

Fig. 15. President Funderburk presents a Certificate of Achievement to Amanda Rebecca Zeiler for winning the Junior Section of FES Special Awards at the 42nd State Science and Engineering Fair of Florida.

Fig. 16. Vicky Buckles receives a Certificate of Achievement for winning the Senior Section of FES Special Awards at the 42nd State Science and Engineering Fair of Florida.

Fig. 17. Liana Glanville accepts the FES Achievement Award for Education (K-12).

All photographs courtesy of Frank Mead.