ON SEXUAL SELECTION IN FLORIDA'S *PYRACTOMENA BOREALIS* (COLEOPTERA: LAMPYRIDAE)

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ABSTRACT

To what extent can a small animal with limited mobility use behavior to take advantage of its environment and how might this influence the population as a whole? This was examined in a firefly species *Pyractomena borealis* (Randall), by looking at the features of the microhabitat where larvae pupate, how developmental rates are influenced by extrinsic factors, and how the population's spatial distribution differed according to sex. In two populations of *P. borealis* in Gainesville Florida, larvae pupated at the warmest locations on trees, potentially causing a faster development rate than individuals in cooler areas. In these populations males pupated sooner and in warmer areas than females, suggesting males chose their pupation locations in order to have a shorter development period and an earlier emergence date than females. This is the first evidence of protandry being experimentally linked with behavioral usage of habitat.

Key words: protandry, Microhabitat, Microclimate, Pupation Duration, Ectotherm, Behavior

RESUMEN

Hasta que punto puede un animal pequeño con mobilidad limitada usar el comportamiento para aprovecharse de su ambiente y como esto puede influenciar la población completa? Esto fué investigado en una especie de luciérnaga *Pyractomena borealis* (Randall), al observar las características del microhabitat donde se empupan las larvas, como las tasas de desarrollo son influenciadas por factores extrínsicos, y como la distribución espacial de la población varia de acuerdo al sexo. En dos poblaciones de *P. borealis* en Gainesville Florida, las larvas se empuparon en las localidad más cálidas de los arboles, potencialmente causando una tasa de desarrollo más rápido que en los individuos en áreas más heladas. En estas poblaciones los machos empuparon más pronto en las áreas más calidas que las hembras, sugeriendo que los machos escogen las localidades donde van a empupar para tener un periodo de desarrollo más corto y una fecha de emergéncia de las hembras más temprana. Esta es la primera evidencia de protandria que experimentalmente conecta el comportamiento del uso del habitat.

Virtually all aspects of the life history of an ectotherm (physiology, development, activity levels, reproduction, etc.) are strongly influenced by ambient temperature (Fagerstrom & Wiklund 1982; Branson 1986; Zonneveld & Metz 1991; Wiklund et al. 1996; Olsson et al. 1999; Hemptinne et al. 2001). Behavioral responses to the limitations of being an ectotherm may be an important factor in the evolution of a species. There is no clearer example of this than the behavior that is involved in pupation.

Arboreal Pupation

Unlike most lampyrids, which pupate underground, members of the genus *Pyractomena* (and perhaps all of the fireflies in the tribe Cratomorphini) pupate above ground, mostly on vegetation (Lloyd 1997). *Pyractomena borealis* (Randall) larvae climb up tree trunks and glue the holdfast organ (at the tip of their abdomens) to the tree trunk (Lloyd 1997; Archangelsky & Branham 1998). Pupae hang upside down, generally with their ventral surface against the tree, the same position they use during ecdysis between larval instars (Archangelsky & Branham 1998).

There are many potential costs associated with arboreal pupation that are not as extreme for species that pupate underground. An underground burrow buffers environmental temperature fluctuation while arboreal pupation provides little shelter from such extremes. Similarly, burrows are moist environments, whereas arboreal pupation presents a greater risk of desiccation. Finally, underground pupae are less exposed to predation and parasitism compared to the often highly visible *P. borealis* pupae. Given these additional costs of arboreal pupation, why should this unusual mode of pupation exist at all?

Lloyd (1997) suggested that *Pyractomena* evolved aerial pupation as a way to avoid floodwaters, since the habitats they are found in are prone to flooding. While arboreal pupation may also expose the firefly to extremes of temperature, they may be exposed to much warmer average temperatures than species that pupate in the ground; thus, there is a potential for more rapid development and earlier eclosion (Regniere et al.

1981; Fagerstrom & Wiklund 1982; Branson 1986; Wagner et al. 1987; Leather 1990; Wiklund et al. 1996; Hemptinne et al. 2001). *P. borealis* is unique in Florida because adults can emerge as early as mid-February.

Protandry

Protandry (males maturing to a reproductive stage earlier than females) occurs commonly in ectotherms and has been found in many insect species (Wiklund & Fagerstrom 1977; Wiklund & Solbreck 1982; Regniere et al. 1981; Fagerstrom and Wiklund 1982; Bulmer 1983a, b; Parker & Courtney 1983; Branson 1986; Zonneveld & Metz 1991; Wedell 1992; Wiklund et al. 1992; Nylin et al. 1993; Wiklund et al. 1996; Zonneveld 1996; Bradshaw et al. 1997; Carvalho et al. 1998; Harari et al. 2000). Protandrous systems have been shown to have sexual advantages for males (Fagerstrom & Wiklund 1982; Zonneveld & Metz 1991; Wedell 1992; Nylin et al. 1993; Harari et al. 2000). Emerging early gives males the advantage of having virgin females to mate with, increased time to produce sperm, and assurance that they will not emerge after the female population begins to decline resulting in either no or low quality females remaining (Wiklund & Fagerstrom 1977; Wiklund & Solbreck 1982; Fagerstrom & Wiklund 1982; Wiklund et al. 1992; Wiklund et al. 1996; Zonneveld 1996b; Carvalho et al. 1998; Olsson et al. 1999). It has also been suggested that females may not merely be passive participants in protandry, but may actually benefit from emerging after males and therefore be selected to do so (Wiklund & Solbreck 1982; Zonneveld & Metz 1991; Wedell 1992; Wiklund et al. 1996). Protandry could reduce the chances of pre-reproductive mortality in females (Wiklund & Solbreck 1982; Zonneveld & Metz 1991; Wedell 1992; Wiklund et al. 1996; Harari et al. 2000) and also act as a mechanism for passive female choice by assuring that females mate with old and therefore, by way of longevity, the fittest males (Wedell 1992).

Protandry has not been reported in any *Pyractomena* species (Buschman 1977), though this may be because it has not been specifically looked for. However it has been suggested that protandry may occur in the firefly *Photinus knulli* (Cicero 1983) and in other *Photinus* species (Lewis & Wang 1991).

P. borealis is vulnerable to extreme temperature variation during pupation. Therefore it is possible that the microhabitat of a pupation site influences the developmental rate of individuals, and if there is a sex difference in microhabitat usage, it is possible this may influence the dynamics of protandry across a population (Regniere et al. 1981; Bulmer 1983a; Fagerstrom & Wiklund 1982; Parker & Courtney 1983; Branson 1986; Leather 1990; Zonneveld & Metz 1991; Wiklund et al. 1992; Nylin et al. 1993; Wiklund et al. 1996; Harari et al. 2000; Hemptinne et al. 2001).

Behavioral manipulation of emergence timing has been suggested by Regniere et al. (1981) for the Japanese beetle (Scarabaeidae: *Popillia japonica*). Since the duration of pupation is dictated by temperature, males might pupate at different soil depths according to surface temperature to decrease pupation duration and to emerge before females. Similarly, there is high variation in the arboreal microhabitats of *P. borealis* and thereby the potential to exploit certain microhabitats. This study looks at how behavioral manipulation of emergence timing by individuals could potentially impact the population dynamics through protandry.

Materials and Methods

The Study Areas

This study was performed in mid-January, 2001 at two locations in Gainesville, Alachua County Florida (Latitude = 29°41'N, Longitude = 82°16'W). Study area A was a flood plain forest located in a residential area between Blues Creek and Devil's Millhopper Geological Site. Study area B was located in Possum Creek, also a flood plain forest. Deciduous trees dominated both habitats. The specific plots were 30.5 m by 30.5 m areas with high concentrations of *P. borealis* larvae. All of the trees in these plots were numbered and categorized according to bark roughness on a scale of 1-5 (1 = the smoothest, 5 = the roughest). Similarly, tree calipers were used to measure all the tree's width (east to west axis) and depth (north to south axis) at 1.22 m from the ground.

Collection Techniques and Measurements

Between 18th of January 2001 (day-of-year 18) and 18th of February 2001 (day-of-year 49) all trees at both plots were scanned daily for attached *P. borealis* larvae, pupae, and adults. Larval collection date was also their attachment date because of the daily scans. Trees were scanned between ground level and up to nine meters. Once an individual was located, I assigned a number to it and recorded the stage (larvae or pupae), tree number it was found on, height above ground, and the aspect of the individual using a Suunto® compass. For this study, aspect is considered the compass direction the individual was facing (i.e. the face of the tree it was on). These data were used to describe the microhabitat, that is, the apparent key features of the specific location at the point of attachment described at the scale that is relevant to that individual. If the individual was within reach, I collected the firefly by scoring the bark approximately 2.5 cm around the individual with a contractor grade Stanley utility blade; the

section of bark was then pried from the tree with a wood-carving chisel. The specimen was placed in a semi-opaque plastic film canister covered with netting secured with a rubber band.

Rearing Temperatures

The specimens were immediately taken back to the laboratory and randomly and evenly distributed amongst three rearing chambers. Two of the chambers were Florida Reach-in Chambers® set at a constant temperature of 13 and 24°C respectively. The other chamber was an Environator® set at a constant temperature of 18°C. All three chambers maintained a constant humidity of 70% and nine hours of light (8 am-5 pm) simulating the natural hours of daylight at the start of the field season. I monitored the fireflies every day and recorded their date of pupation and eclosion, sex, and adult weight.

Field Temperature Monitoring

At study area A the ambient temperature was monitored on eleven trees randomly selected within the marked plot. I refer to these data as the microclimate measurements, not to be confused with the microhabitat data collected for individual pupation locations. Microhabitat is defined by the features of a specific location (tree size, aspect, height, bark roughness); microclimate in this study is considered the temperature regime for a specific point on a tree.

I used four Optic StowAway® Temp loggers (Onset Computer Corporations, Bourne, MA) on each tree to measure microclimate. The loggers were placed at 0°N and 0.61 m above ground, 0°N and 2.44 m above ground, 180°S and 0.61 m above ground, and 180°S and 2.44 m above ground. The loggers recorded the temperature every 30 seconds for 66 hours.

I used two approaches to analyze the temperature data. The first was to find the mean hourly temperature and standard deviation (as a measure of temperature variability within hours) for each location. As successive temperature readings are not truly independent, for the second method of analysis, I randomly selected five percent of the total recorded data. The random selection increased the independence of the individual temperature readings. This process was repeated ten times to ensure accurate representation of the data by the random selection. In this case, there was no measurement of standard deviation.

Statistics

I conducted the statistical analyses using SPSS version 9.0® (SPSS Inc., Chicago, Illinois). All data sets were examined for normality using a Kolmogorov-Smirnov test. When data were normally distributed, or could be transformed to be normally distributed, I utilized parametric tests for subsequent analyses. I analyzed non-normal data using appropriate non-parametric tests. The specific tests used are detailed in the results section.

Results

The Habitat Data

To ensure equality of tree distribution between the two sites, I first had to compare the size of the trees. Tree width and depth were not normally distributed at either study area. There was no difference in tree width between study area A and B (Mann-Whitney U = 6099, Z = -1.191, p = 0.234) and no difference in depth (Mann-Whitney U = 6148.5, Z = -0.998, p = 0.318).

In order to find physical characteristics of a microhabitat that would influence the microclimate, I analyzed the mean and the standard deviation of the hourly temperature for each microhabitat. The mean and standard deviation of the hourly temperature were not normally distributed. I developed a stepwise linear regression model using the mean hourly temperature as the independent variable to examine the potential causes of temperature variation. The putative explanatory variables entered were the vertical height up the tree, the side on the tree (North = 0°, South = 180°), the tree width (representing the tree's girth), the bark roughness, the day of the year, how many hours from noon it was, and whether it was AM or PM. I split these data into the two latter variables for analysis to reduce the circular nature of time.

All significant variables had positive correlations with the mean hourly temperature. Beginning with the most significant, these variables were: The time of day according to the number of hours from noon (Adjusted R^2 = 0.285, Pearson Correlation = 0.533, F Change = <0.001), the day of the year (Adjusted R^2 = 0.184, Pearson Correlation = 0.434, F Change = <0.001), if the sample was taken in the AM or the PM (Adjusted R^2 = 0.101, Pearson Correlation = 0.357, F Change = <0.001. A positive correlation means that it was warmer in the PM), the size of the tree (Adjusted R^2 = 0.061, Pearson Correlation = 0.138, F Change = <0.001), or if the microhabitat was facing north or facing south (Adjusted R^2= 0.003, Pearson Correlation = 0.053, F Change = <0.001. The positive correlation meaning that the south was warmer than the north). These variables explained a total of 63.2% of the variation in the mean hourly temperature.

I repeated the same regression model, but used data from 5% randomly selected temperatures as the dependent variable for all ten replicates. The mean adjusted R^2 value for these ten trials was 0.622, and the standard deviation

0.003. In all ten cases the same variables occurred in the same order as the hourly mean values. However, in four out of ten trials the height up the tree was included as the last variable in addition to the other five variables. The mean adjusted R^2 change when adding the height variable was less than 0.001.

To examine the potential causes of the variation in the fluctuation of temperature, I conducted a stepwise linear regression using the square root of the standard deviation of the mean hourly temperature. The square root of the standard deviation was used as the dependent variable to make the data more normally distributed. The independent variables included for analysis were the same as the stepwise linear regression of the mean temperatures.

In order of significance, the variables with a positive correlation to the variance of the hourly mean temperature were: The time of day according to the number of hours from noon (Adjusted R^2 = 0.353, Pearson Correlation = 0.594, F Change = <0.001), if the microhabitat was facing north or facing south (Adjusted R^2 = 0.017, Pearson Correlation = 0.135, F Change = <0.001. The positive correlation means the south was more fluctuating than the north), if the sample was taken in the AM or the PM (Adjusted R^2 = 0.016, Pearson Correlation = 0.195, F Change = <0.001) A positive correlation means that it was more fluctuating in the PM), the day of the year (Adjusted R^2 = 0.004, Pearson Correlation = 0.061, F Change = <0.001), and the bark roughness (Adjusted R^2 = 0.003, Pearson Correlation = 0.024, F Change = <0.001). The size of the tree was negatively correlated with the variance of the mean hourly temperature (Adjusted R^2 = 0.013, Pearson Correlation = -0.041, F Change = <0.001). These variables explained a total of 40.4% of the variation in the variance of the mean hourly temperature.

Distribution of Fireflies at Study Areas A and B

I compared the physical characteristics of those trees with and without fireflies to determine any differences between the trees fireflies "chose" to pupate on and those they did not. Trees with fireflies were larger than trees without fireflies (Width: Mann-Whitney U = 2777.5, Z = -7.224, p < 0.001; Depth: Mann-Whitney U = 2814.5, Z = -7.101, p < 0.001). Trees with fireflies were also rougher than trees without fireflies (Chi square = 12.7, p < 0.01, df = 3).

To examine differences between the distribution of males and females, I analyzed height, girth, and aspect of pupation locations with respect to sex. Males were found higher up the trees than females (Mann-Whitney U = 2230, Z = -2.148, p = 0.032). Males were also found on larger trees than females (Width: Mann-Whitney U = 2301, Z = -1.993, p = 0.046; Depth: Mann-Whitney U = 2287.5, Z = -2.044, p = 0.041). Females deviated more from 180° than males did, i.e. males were more clustered on the south side of the trees than females (Mann-Whitney U = 2250.500, Z = -2.071, p = 0.038) (see Fig. 1 for females, and Fig. 2 for males). The descriptive statistics for the distribution of female and male *P. borealis* can be found in Tables 1 and 2, respectively.

Attachment Timing

I looked at the population wide pattern of development in order to begin examining protandry in *P. borealis*. The collection dates of the larvae (i.e. attachment dates, expressed as Day-of-Year or DY. January 1st is 1 DY, February 1st is 32 DY) were not normally distributed. Overall, females were collected and therefore had attached later than males (Females: N = 70, Mean = 28.21 DY, Median = 27 DY, SD = 7.13; Males: N = 81, Mean = 23.84 DY; Median = 22 DY, SD = 5.36; Mann-Whitney U = 1464, Z = -3.915, p < 0.001).

Developmental Timing According to Ambient Temperature

I compared the development rates for individuals reared under the three different temperature regimes to determine temperature effect on pupation. None of the developmental parameters that were measured were normally distributed. The duration of the attached larval stage, pupation, and emergence all decreased with increasing temperature (see Tables 3 and 4). The general descriptive statistics for all variables at 13°C, 18°C, and 24°C can be found in table 4.

In all three temperature regimes females pupated and also emerged as adults on later dates

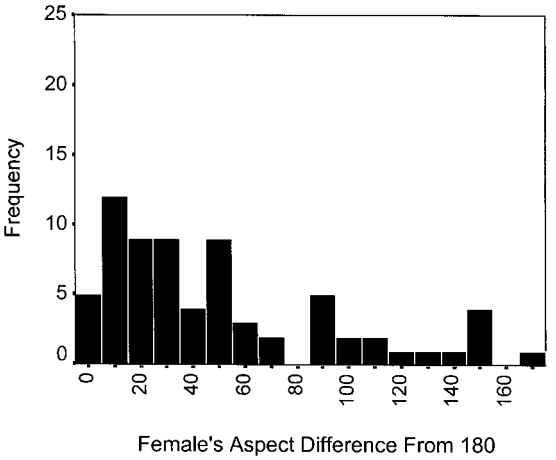

Fig. 1. The Female's Aspect Deviation from 180°. On the X axis 0 represents south, because it is the difference from 180.

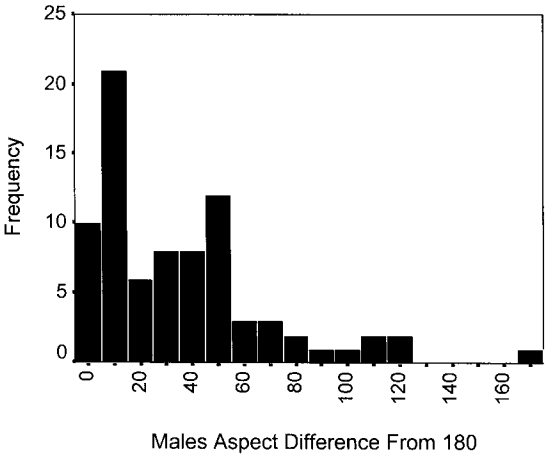

Fig. 2. The Male's Aspect Deviation from 180°. On the X axis 0° represents south, because it is the difference from 180°.

than males (see Tables 5, 6, and 7). At 13°C and 24°C the length of time it took from attachment to pupation was longer in females (see Tables 5 and 7). However, at 18°C and 24°C the length of pupation was longer for males than for females (see Tables 6 and 7). At 13° the total length of time from collection to emergence was significantly longer in females (see Table 5). The descriptive statistics for all of the significant results are found in Table 8.

To examine the potential causes of variation in the total duration of development, from attached larvae to eclosion, I developed a stepwise linear regression model using the total number of days from collection to emergence as the independent variable. Date of collection, rearing temperature, sex, and adult weight were entered as the possible explanatory variables. The temperature the individual was reared at was negatively correlated with the duration of development (Adjusted $R^2 = 0.748$, Pearson Correlation = -0.865, F Change = <0.001). The sex of the individual was positively correlated with the duration of the development; meaning that individuals with longer development times tended to be female (Adjusted $R^2 = 0.016$, Pearson Correlation = 0.287, F Change = 0.003). These two variables explained 76.0% of the total variation of development times.

Discussion

In this study I have shown that *P. borealis* tends to pupate in the warmest microhabitats and that warmer temperature leads to faster pupation rates. In addition there were temporal and spatial differences between males and females. Males not only attach earlier than females, but they also pupate in warmer areas than females. These two behaviors would lead to males emerging earlier than females; this suggests that protandry is found in *P. borealis* and the degree of protandry in a population may be influenced by the behavior of individuals.

Microhabitat Features

The largest features in the variation of temperature were not surprisingly associated with time. The first three features were related to the time of day and the day of the year. However, tree size and the aspect of attachment were also significantly important contributors to the variation of mean hourly temperature. Larger trees were warmer than smaller trees; large trees retain absorbed heat from the sun more than smaller trees. This was also shown by Lloyd (1997) through his physical model experiment that simulated different microhabitats that *P. borealis* might encounter. In addition, the south side of the tree was warmer than the north side. This result is also expected because the south side of the tree receives direct sunlight (and therefore solar radiation) where the north side does not. This also corresponds with the results of Lloyd's physical models (1997).

Interestingly, height was not a feature that influenced the variation of mean hourly temperature between microhabitats. This seemingly contradicts the results of Lloyd's (1997) model trees that found height to be positively correlated with temperature. This result may also be due to half of the data coming from the north side, therefore the data with significant differences in height from the south side would had less of an influence on the data set as a whole. However, upon closer examination,

Table 1. Descriptive statistics of female distribution.

	N	Mean	Std. deviation	Percentiles		
				25th	50th (median)	75th
Deviation from 180° aspect	70	49.971	44.559	14.50	37.00	74.50
Height up tree (m)	70	1.625	0.574	1.120	1.646	2.073
Tree width (m)	70	0.203	0.137	0.086	0.180	0.318
Tree depth (m)	70	0.200	0.133	0.086	0.180	0.326

TABLE 2. DESCRIPTIVE STATISTICS OF MALE DISTRIBUTION.

	N	Mean	Std. deviation	Percentiles		
				25th	50th (median)	75th
Deviation from 180° aspect	80	34.738	34.060	5.250	26.500	52.250
Height up tree (m)	80	1.818	0.592	1.379	1.905	2.240
Tree width (m)	81	0.245	0.139	0.131	0.216	0.318
Tree depth (m)	81	0.244	0.137	0.127	0.218	0.326

when viewed at a tree-by-tree basis, Lloyd's findings are in fact corroborated by this study. Height was important in four out of the ten trials examining 5% of the randomly selected data. This may reflect the inconsistent nature of solar exposure to trees in the same forest. Not all trees are in areas of uniform solar exposure; therefore on some trees height is an important feature for maximizing heat. The randomly selected data would not contain an equal representation of all trees, so those trees in areas of patchy sunlight where height was important may have had a larger representation in the four trials where height was important.

Time of day also plays a key role in the fluctuation of temperature, but the second most important feature is the aspect. Areas on the south side of the tree fluctuate much more than areas on the north side; the north side continuously being in shadow, and the south side receiving more or less solar radiation depending on cloud cover, shadows, etc. . . . Finally, tree size is negatively correlated with temperature fluctuation; larger trees have more stable microclimates than smaller trees. This is corroborated by Lloyd's study of model trees (1997). This is probably for similar reasons as to why large trees are warmer, because large trees have a smaller surface to volume ratio, they can maintain absorbed heat longer than small trees, therefore making them more stable.

The contribution of microhabitat features on the microclimate may appear to be minor, but it is important nonetheless (Ohsaki 1986). All fireflies are exposed to the same daily and seasonal effects of temperature, but aspect, tree size, and height are all features that individuals can control through behavioral decisions. An individual that has selected to pupate on the south side of a large tree will, over the course of several days, have the advantage because of the cumulative effect of the warmer temperature throughout development. If this behavior were genetically based, it would be a source of selectable variation among individuals.

Pupation Site Selection Behavior Based on Microhabitat

It is important to note that there was no significant difference of tree characteristics between the two sites, therefore we may assume the microclimate data collected for study area A can also be applied to study area B. The overall distribution of *P. borealis* suggests that the fireflies are taking advantage of the best microhabitats to maximize the temperature of their microclimate. Trees with fireflies were larger and had rougher bark than trees without fireflies. This study also confirms Lloyd's findings in 1997 that *P. borealis* prefer the south side of the tree, but does not support his findings that individuals preferred smoother trees; this difference may be due to differences in habitats and the availability of bark types. Height also seemed to be an influencing factor; as suggested by Lloyd (1997), *P. borealis* pupate higher than is necessary to avoid floodwaters. On some trees this may take advantage of areas with more direct sunlight. The features that determined the distribution of *P. borealis* were also the same features that maximized the mean temperature.

The distribution of *P. borealis* stands in stark contrast to that of *P. limbicollis*. *P. limbicollis* pupate low to the ground on the northeastern side of small trees; they also emerge several weeks after *P. borealis* (Lloyd 1997). The distribution of *P. limbicollis* suggests that these fireflies are in fact taking advantage of the cooler more stable envi-

TABLE 3. COMPARISONS OF DEVELOPMENT AMONG INDIVIDUALS REARED AT 13, 18, AND 24°C. IN ALL CASES, THE VALUES FOR 13°C ARE LARGER THAN 18°C, WHICH IS LARGER THAN 24°C.

	Kruskal-Wallis	df	p
Pupation date	31.081	2	<0.001
Emergence date	94.496	2	<0.001
Attached larvae duration	67.659	2	<0.001
Pupa duration	105.868	2	<0.001
Attached larvae to emergence duration	110.300	2	<0.001

TABLE 4. DESCRIPTIVE STATISTICS OF DEVELOPMENT AT THREE DIFFERENT TEMPERATURES. THE PUPATION AND EMERGENCE DATES ARE IN UNITS OF THE DAY-OF-YEAR (DY). THE ATTACHED LARVAE DURATION, PUPA DURATION, AND LARVAE TO PUPA DURATION ARE IN UNITS OF NUMBER OF DAYS (D).

	Temperature	N	Mean	Std. deviation	Percentiles 25th	50th (median)	75th
Pupation date (DY)	13°C	40	42.38	11.60	32.00	45.00	51.00
	18°C	45	34.44	8.22	26.50	36.00	42.00
	24°C	54	29.67	6.97	24.00	28.50	33.25
Emergence date (DY)	13°C	37	76.57	15.60	67.00	78.00	88.00
	18°C	49	48.57	7.62	42.00	49.00	55.50
	24°C	56	36.68	6.90	31.00	35.00	41.00
Attached larvae	13°C	40	16.93	7.24	10.25	17.50	24.00
Duration (D)	18°C	45	8.07	4.45	5.00	8.00	11.00
	24°C	54	4.33	2.07	3.00	4.00	6.00
Pupa duration (D)	13°C	35	35.83	6.23	35.00	36.00	37.00
	18°C	44	15.93	7.06	13.00	14.00	16.00
	24°C	53	7.36	0.56	7.00	7.00	8.00
Larvae to emergence	13°C	37	51.22	11.60	45.00	54.00	60.00
Duration (D)	18°C	49	21.41	5.42	17.50	22.00	25.00
	24°C	56	11.38	2.40	10.00	12.00	13.00

ronments (the lower stability of small trees is probably counterbalanced by the preference for the north side). In this case, they would also not need to pupate high up the trees to maximize light, but merely high enough to avoid flood waters (Lloyd 1997). *P. limbicollis* is considerably smaller than *P. borealis*, so it may be that *P. limbicollis* is too small to overcome the potentially desiccating effects of direct sunlight.

Developmental Timing According to Ambient Temperatures

All insects have a temperature threshold above which they can develop, and warmer temperatures cause faster development rates in insects (Regniere et al. 1981; Branson 1986; Wagner et al. 1987; Leather 1990; Miller 1992; Wiklund et al. 1996; Hemptinne et al. 2001); *P. borealis* is no exception. In this study, pupal development was shown to be shorter at warmer temperatures, and temperature was the largest influence on development time. This suggests that the selective behavior of *P. borealis* to pupate in microhabitats with the warmer microclimates will result in reduced developmental durations. The laboratory conditions *P. borealis* were reared in were conservative compared to the actual field sites. This suggests there may be more highly variable development rates based on microclimate in the field than were seen in the laboratory.

Protandry

Protandry is evident in *P. borealis*. In the field males attach before females. In the laboratory males pupate and emerge earlier than females. However, there are differences at each of the three temperatures, suggesting that the patterns of development of the sexes are not consistent throughout a wide range of temperatures. At 13°C females have a longer development time, but at 18 and 24°C there is no difference in the total development time between the sexes at $p < 0.05$. However, sex was a determinant of developmental duration in the linear regression; meaning an individual with a long developmental time would most likely be a female.

TABLE 5. COMPARISONS OF THE DEVELOPMENTAL STAGES BETWEEN MALES AND FEMALES REARED AT 13°C.

	Mann-Whitney U	Z	p	Value higher for (see real numbers in Table 8)
Pupation date	54.50	-3.44	0.001	Female
Emergence date	57.50	-3.34	0.001	Female
Attached larvae duration	54.00	-3.46	0.001	Female
Pupation duration	94.00	-1.72	0.086	Not significant
Larvae to emergence duration	62.00	-3.19	0.001	Female

TABLE 6. COMPARISONS OF THE DEVELOPMENTAL STAGES BETWEEN MALES AND FEMALES REARED AT 18°C.

	Mann-Whitney U	Z	p	Value higher for (see real numbers in Table 8)
Pupation date	74.5	-2.67	0.008	Female
Emergence date	113.0	-2.70	0.007	Female
Attached larvae duration	109.5	-1.55	0.121	Not significant
Pupa duration	70.0	-3.07	0.002	Male
Larvae to emergence duration	176.0	-1.11	0.266	Not significant

This latter result is consistent with studies of *P. lucifera* in which females have a longer duration of the larval stage and therefore males pupate sooner than females (Buschman 1977). Interestingly, in both of these systems, the actual duration of the pupal stage is longer for males than for females (Buschman 1977). The explanation for this extended pupation duration is unknown. However, regardless of the developmental differences there has been no suggestion of protandry in *P. lucifera* (Buschman 1977). Protandry may be limited in this system because the male's slow pupation duration negates any time advantage they gained by attaching early.

Microhabitat Influences on Protandry

When looking at all the individuals collected, there is a significant difference between the pupation locations of males and females. Overall, males were found on larger trees and were located on the south side more often and were higher up the trees. From what we know about microhabitat, the males appear to be maximizing developmental rates through microclimate more than the females.

It is unclear whether the females are "intentionally" choosing smaller trees, lower down and deviating from the south more than males in order to slow their development or are simply choosing a "large enough" tree without using up time looking for the largest tree to pupate on. It may also take more effort to find the southern most part of a tree, and so females may not be that specific in their site selection to save time and energy. Similarly, it was shown on some trees that height positively influences microclimate and so it is also unclear if females are specifically selecting low pupation sites on the trees or if they are pupating just high enough for successful development. In contrast, the behavior of males seems to have an obvious result. By pupating on large trees on the southern-most part and pupating significantly higher than females males can take advantage of microhabitat to decrease their development time.

In *P. borealis* there is an obvious benefit to males that emerge early, it gives them more time to search for adult females and more time to "tend" pupae and mate with eclosing females (Lloyd 1997). The benefits for females are not as evident. Many have suggested that females can benefit from protandry through reducing premating mortality (Wiklund & Solbreck 1982; Fagerstrom & Wiklund 1982; Zonneveld & Metz 1991; Wedell 1992; Wiklund et al. 1996; Harari et al. 2000). However, females seem more vulnerable as immobile pupa than as mobile adults and so it is unclear why they would want to prolong this stage. It has also been suggested that females benefit from protandry through passive female choice (Wedell 1992). However, because the pupal "tending" behavior by males is greatly enabled by protandry, the benefits of female passive choice must be considered in light of the costs associated with being "tended" as a pupa.

Previously published models that discuss protandry suggest that developmental timing is primarily under physiological control (Wiklund & Fagerstrom 1977; Wiklund & Solbreck 1982; Regniere et al. 1981; Parker & Courtney 1983; Branson 1986; Zonneveld & Metz 1991; Nylin et al. 1993; Bradshaw et al. 1997). In the case of *P. borealis*, developmental timing is influenced by behavior with regard to the choice of pupation site.

TABLE 7. COMPARISONS OF THE DEVELOPMENTAL STAGES BETWEEN MALES AND FEMALES REARED AT 24°C.

	Mann-Whitney U	Z	p	Value higher for (see real numbers in Table 8)
Pupation date	12.500	-2.617	0.009	Female
Emergence date	14.500	-2.599	0.009	Female
Attached larvae duration	17.500	-2.307	0.021	Female
Pupa duration	22.000	-2.288	0.022	Male
Larvae to emergence duration	26.500	-1.887	0.059	Not Significant

TABLE 8. DESCRIPTIVE STATISTICS OF DEVELOPMENT AT THREE DIFFERENT TEMPERATURES BETWEEN MALES AND FEMALES. THE PUPATION AND EMERGENCE DATES ARE IN UNITS OF THE DAY-OF-YEAR (DY). THE ATTACHED LARVAE DURATION, PUPA DURATION, AND LARVAE TO PUPA DURATION ARE IN UNITS OF NUMBER OF DAYS (D).

	Temperature	Sex	N	Mean	Std. deviation	Percentiles		
						25th	50th (median)	75th
Pupation date (DY)	13°C	Female	9	40.00	9.54	32.50	36.00	51.00
		Male	12	32.92	8.35	26.25	31.00	39.00
	18°C	Female	21	38.10	7.74	32.50	38.00	44.00
		Male	15	30.87	7.04	25.00	30.00	37.00
	24°C	Female	21	33.10	7.06	27.00	33.00	37.50
		Male	33	27.49	6.063	23.00	25.00	29.50
Emergence date (DY)	13°C	Female	9	74.78	9.50	67.00	71.00	85.50
		Male	12	65.50	15.85	62.00	67.00	76.25
	18° C	Female	22	51.96	7.43	48.25	51.50	57.25
		Male	20	45.60	6.68	41.00	45.50	51.75
	24°C	Female	21	40.19	6.88	35.00	40.00	44.50
		Male	35	34.57	6.07	31.00	32.00	37.00
Attached larvae	13°C	Female	9	15.11	5.47	11.50	14.00	19.50
Duration (D)		Male	12	11.42	6.27	7.00	9.50	17.00
	18°C	Female	21	9.10	4.70	5.50	9.00	13.00
		Male	15	6.67	3.60	5.00	6.00	9.00
	24°C	Female	21	5.62	1.75	4.00	6.00	7.00
		Male	33	3.52	1.86	2.00	4.00	5.00
Pupa duration (D)	18°C	Female	21	13.62	1.36	13.00	13.00	14.00
		Male	16	19.69	10.76	14.00	16.00	16.75
	24°C	Female	21	7.10	0.44	7.00	7.00	7.00
		Male	32	7.53	0.57	7.00	7.50	8.00
Larvae to emergence	13°C	Female	9	49.89	5.30	46.00	49.00	54.50
Duration (D)		Male	12	44.25	14.25	42.25	45.50	53.75

It is clear that future models should also consider behavior as a mechanism for protandry.

This study is the first to experimentally link protandry with behavioral usage of the environment. The variation in microhabitat and its potential effects on individual success provide a basis upon which selection can occur (Regniere et al. 1981; Parker & Courtney 1983). This suggests that fine scale variations in the environment can influence the dynamics of protandry and sexual selection in the population as a whole.

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THE EVOLUTION OF INSECT MATING STRUCTURES THROUGH SEXUAL SELECTION

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ABSTRACT

Mating structures are of interest to a wide range of biologists because, in many taxa, mating structures are incredibly diverse and range widely in elaboration even between closely related species. As a result of this diversity, mating structures have been useful in species identification. Historically, the evolution of diverse mating structures has been attributed to post-zygotic selection for pre-zygotic isolation to avoid production of hybrid offspring. More recently, sexual selection has been proposed as an alternative explanation for the rapid diversification of mating structures. Mating structures could diversify between populations through sexual selection if sexual selection acted differently on mating structures in different populations. Eberhard (1985) wrote a comprehensive book explaining how sexual selection could result in the diversification of mating structures and providing examples to support the hypothesis, but none of the examples were experimental tests of the hypothesis. Since 1985, a few studies have experimentally tested this hypothesis. However, there have been no empirical studies that connect intraspecific selection with interspecific diversification. In this paper, I review the reproductive isolation and sexual selection hypotheses and two recent experimental tests of the sexual selection hypothesis. Then, I provide a description of a system that may allow one to establish a connection between sexual selection on mating structures within a species and diversification of mating structures between species.

Key Words: genitalia, diversification, sexual selection, *Melanoplus*

RESUMEN

Las estructuras de apareamiento son de interes de una amplia variedad de biólogos por que, en muchos taxa, las estructuras de apareamiento son increiblemente diversas y se extiende ampliamente en elaboración aun entre especies estechamente relacionadas. Como resultado de esta diversidad, las estructuras de apareamiento han sido útiles en la identificación de especies. Históricamente, la evolución de las estructuras de apareamiento diversas ha sida atribuida a la selección poscigótico para el aislamiento precigótico para evitar la producción de descendientes híbridos. Más recientemente, la selección sexual ha sido propuesta como una explicación alternativa para la diversificación rápida de las estructuras de apareamiento. Las estructuras de apareamiento puede diversificar entre poblaciones por medio de la selección sexual si la selección sexual actua diferentement en las estructuras de apareamiento en poblaciones diferentes. Eberhard (1985) escribió un libro comprehensivo explicando como la selección sexual puede resultar en la diversificación de las estructuras de apareamiento y proveyendo ejemplares para apoyar su hipótesis, pero ninguno de los ejemplares fueron pruebas experimentales de la hipótesis. Desde 1985, unos pocos estudios han probados experimentalmente esta hipótesis. No obstante, no han habido estudios empíricos que relacionan la selección intraspecífica con la diversificación interspecífica. En este papel, examino las hipótesis del aislamiento reproductivo y la selección sexual y dos pruebas experimentales recientes de la hipótesis de la selección sexual. Después, proveo una descripción de un sistema que puede permitir establecer una conección entre la selección sexual de las estructuras sexuales dentro de una especie y la diversificación de las estructuras de apareamiento entre especies.

Morphological structures involved in coupling, and in transferring and receiving sperm have long been of interest to taxonomists because of their utility in distinguishing between species (e.g., Hubbell 1932; Kennedy 1919). These structures (hereafter called "mating structures") are also of interest to evolutionary biologists for two reasons (e.g., Alexander & Otte 1967; Arnqvist 1998; Eberhard 1985; Tatsuta & Akimoto 2000). First, they are much more complex in appearance than would seem necessary for mating. For example, in the damselfly genus *Argia*, male genitalia vary from rather simple structures to extremely complex structures (Fig. 1). It seems unlikely that the difficulty of transferring sperm would differ enough between species in this genus to account for the differences in complexity in genitalia. Second, mating structures show much more rapid diversification than structures that are not involved in mating. This is again exemplified by the diversity

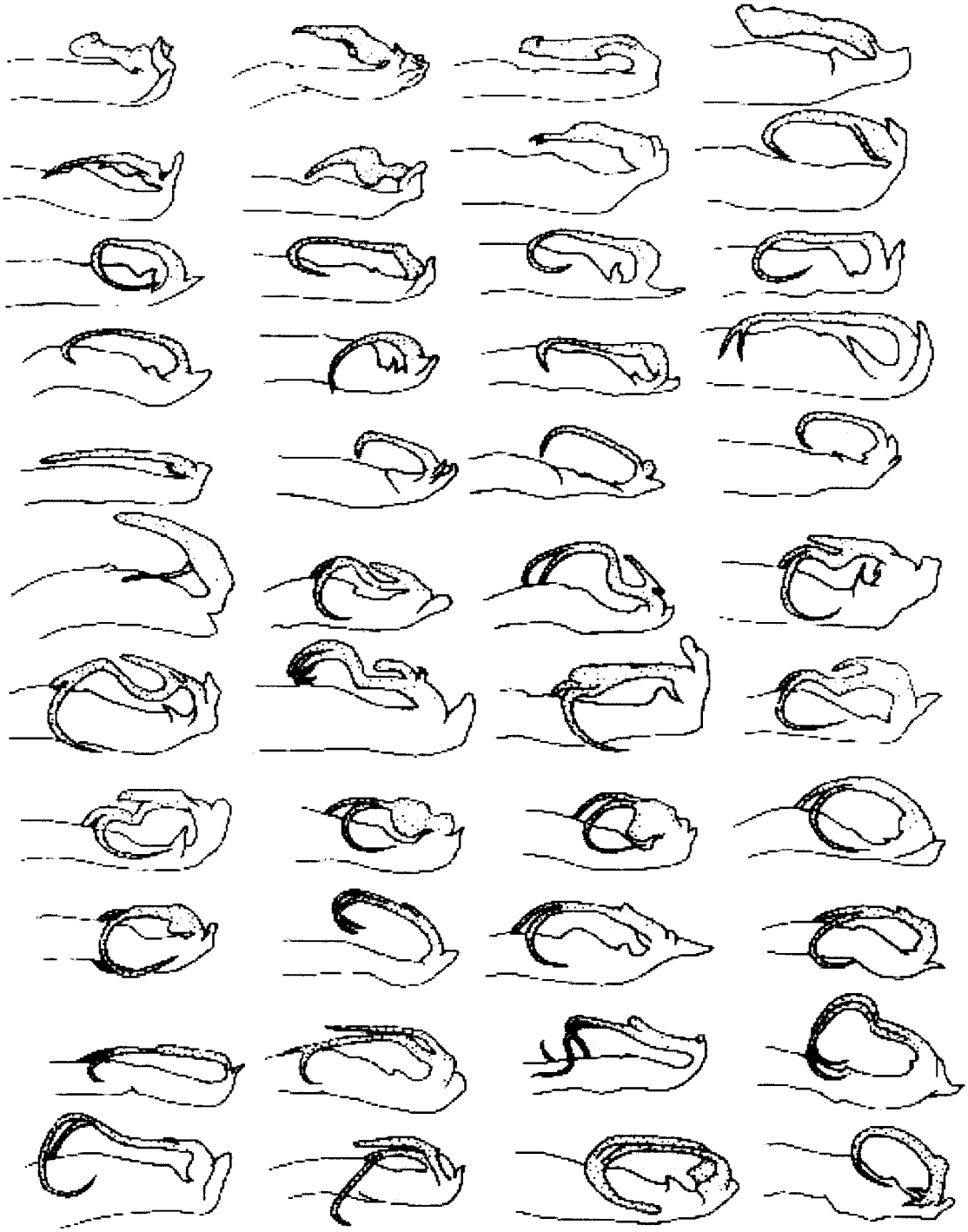

Fig. 1. Diversity of male genitalia in the damselfly genus *Argia*. Figure from Eberhard 1985, reprinted with permission of author and courtesy of Harvard University Press.

of forms of genitalia within the genus, *Argia* (Kennedy 1919; Fig. 1). Similar diversification occurs in female structures that receive and store sperm (e.g., grasshoppers: Slifer 1943; water striders, *Gerris*: Andersen 1993; Fig. 2). Further, we also see this diversification in other structures that are involved in matings such as modified antennae and legs that males use to grasp females

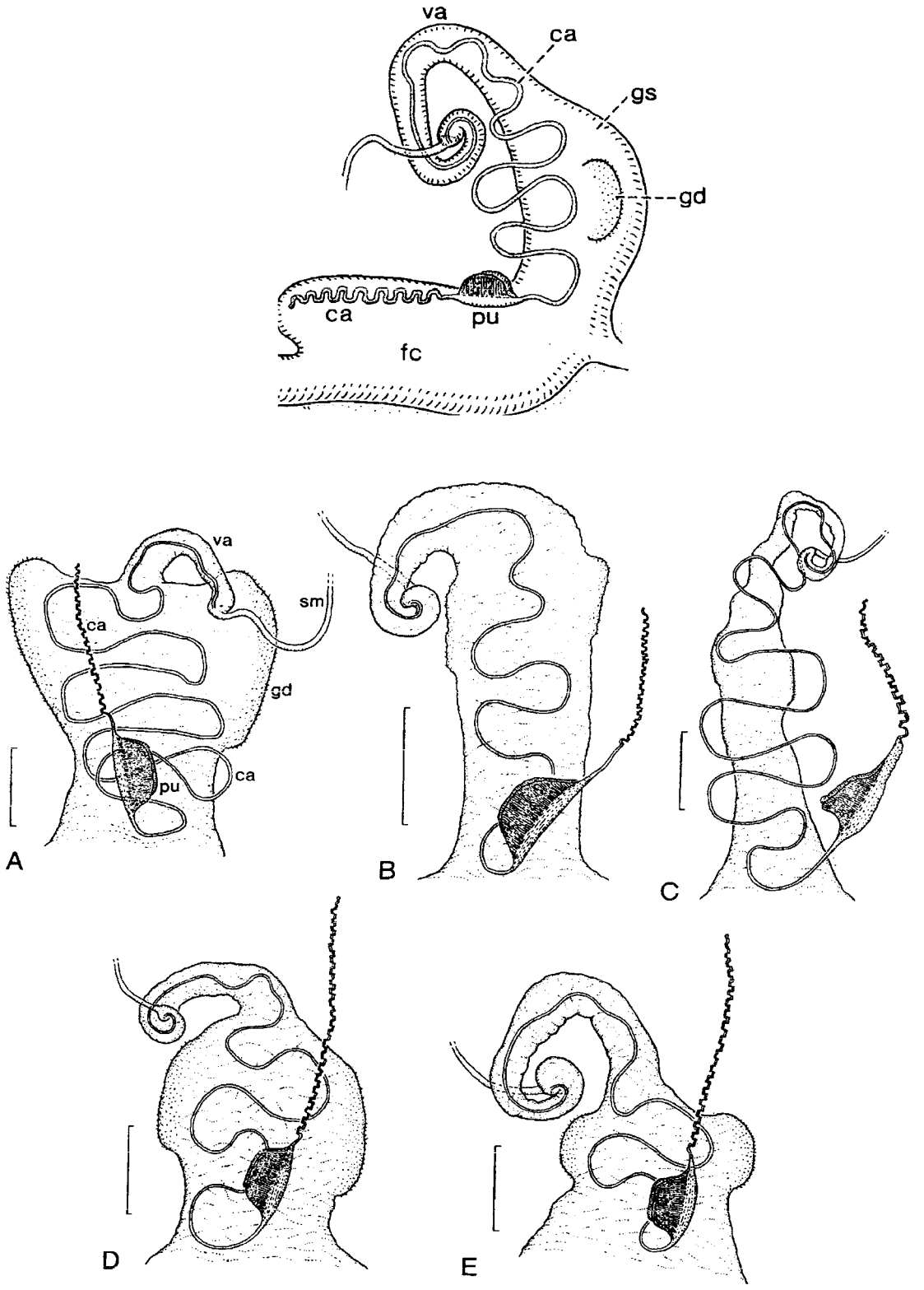

Fig. 2. Diversity of female structures that receive and store sperm in the water strider genus *Gerris*. Drawings from Andersen 1993, reprinted courtesy of the canadian Journal of Zoology.

during copulation in some water strider species. This diversification is seen across many taxonomic groups (Eberhard 1985). Two main hypotheses have been proposed to explain the diversification of genitalia: the reproductive isolation hypothesis and the sexual selection hypothesis.

Reproductive Isolation Hypothesis

Historically, diversification of mating structures has been attributed to selection for prezygotic isolating mechanisms that prevent hybridization. According to this "reproductive isolation" hypothesis (a.k.a. "lock-and-key"), there is strong selection on females to avoid mating with heterospecific males. As a result, females evolve complicated reproductive structures that allow them to discriminate between conspecific and heterospecific males and to avoid heterospecific fertilizations. The occurrence of this process with each speciation event would result in a pattern of rapid diversification of genitalia across closely related species.

The reproductive isolation hypothesis has two main predictions. First, if the diversification and elaboration of mating structures results from selection for reproductive isolation, there should be species-specific fits of male and female mating structures. Second, there should be more diversification of mating structures in sympatry than in allopatry. Certain systems are consistent with these predictions (Eberhard 1985). However, there are many systems for which we do not see a species-specific fit between male and female mating structures; in these species, female structures do not prevent intromission by males of other species (Eberhard 1985; Shapiro & Porter 1989). This finding alone is not sufficient to reject the reproductive isolation hypothesis because it is possible that (1) reproductive isolation is achieved not through a mechanical fit but through a sensory fit such that the male reproductive parts stimulate females in a species-specific manner or (2) the genitalia no longer serve as reproductive isolating mechanisms because other mechanisms have evolved (e.g., behavioral).

Data from many taxa also do not support the second prediction of the reproductive isolation hypothesis. In several cases, rapid diversification of mating structures appears to have occurred in allopatry. There are patterns of extreme diversification of mating structures of species that are geographically isolated from any morphologically similar species. For example, certain species of the homopteran genus *Oliarus* appear to have evolved separately on different islands of the Galapagos (Fig. 3). The male intromittent organs of species on different islands have diverged substantially (Eberhard 1985; Fennah 1967). In sum, there are many cases of apparent rapid diversification of mating structures that the reproductive isolation hypothesis cannot explain.

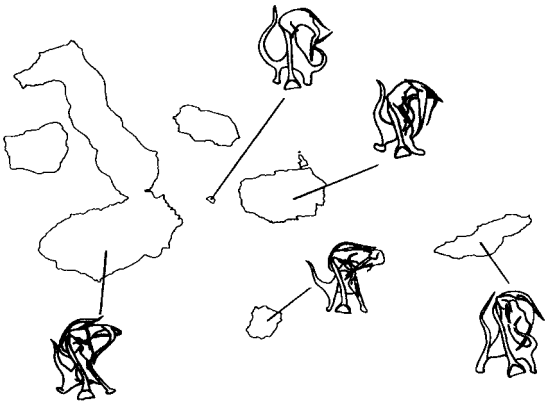

Fig. 3. Male genitalia of species of the homopteran genus *Oliarus* found on different islands of the Galapagos. Figure from Eberhard 1985, reprinted with permission of author and courtesy of Harvard University Press. Genitalia drawings from Fennah 1967, reprinted courtesy of the California Academy of Sciences.

Sexual Selection Hypothesis

An alternative to the reproductive isolation hypothesis is that the diversification of mating structures is a result of sexual selection. Sexual selection results from differential access to mates based on differences in phenotypic traits. However, in the last twenty years, it has become abundantly clear that sexual selection does not end once coupling has begun. Within the female reproductive tract, there are battles between sperm of different males and differential use of sperm by females (Birkhead & Moller 1998; Eberhard 1996). Sexual selection could act on mating structures if differences in the shape or size of these structures resulted in differential coupling and fertilization success (Lloyd 1979; Short 1979). The sexual selection hypothesis is that sexual selection acting on mating structures differently in different populations could result in diversification of mating structures between populations.

There are three mechanisms by which sexual selection can act, and all have been invoked in explaining the evolution of elaborate mating structures. First, sexual selection could act on mating structures through mate choice. Male mating structures may evolve through cryptic female choice in which females preferentially use sperm from males based on characteristics of the male structures. Selection could also act on females, favoring those that have structures that enable them to be more selective amongst males.

Second, sexual selection could act on mating structures through intrasexual competition. For example, selection could act if certain characteristics of male reproductive structures made them better able to deliver sperm or remove or otherwise compete with the sperm of other males.

Third, sexual selection could act on mating structures through intersexual conflict over fertilization. If male quality varies, then females should be selected to choose sperm of high quality males. Males should be selected to overcome the female choice mechanisms and to manipulate female behavior to their advantage (Gavrilets et al. 2001; Holland & Rice 1999; Rice 1996) and selection should act on females to avoid this manipulation (at least to some degree; Alexander et al. 1997; Cordero & Eberhard 2003) leading to an intersexual arms race involving the mating structures of males and females.

Diversification of mating structures between populations through sexual selection is most likely to occur through female choice because female choice can act on arbitrary traits (Andersson 1994). Advances in the study of the evolution of mating structures through sexual selection have taken two forms: investigations of the form and function of mating structures (e.g., Arnqvist 1998; Arnqvist & Thornhill 1998; Eberhard 1992, 2001; Eberhard & Pereira 1993; Fritz & Turner, 2002; Robinson & Novak 1997; Waage 1979) and experimental tests of selection acting on these structures (e.g., Arnqvist and Danielsson 1999; Arnqvist et al. 1997; Cordoba-Aguilar 1999).

Studies of the Form and Function of Mating Structures

Investigations into the form and function of male and female mating structures support the hypothesis that sexual selection is acting on mating structures. For example, in Waage's (1979) classic work on jewelwinged damselflies, *Calopteryx maculata*¸ he concluded that the intricate structures of the damselfly penis were used not only to transfer sperm to females but also to remove sperm of other males from the female reproductive tract. Waage (1979) came to this conclusion based on four lines of evidence. 1. Females who had previously mated had more sperm in their reproductive tract before and after a second mating than when mating was interrupted. 2. When copulating pairs were dissected (after being killed), male genitalia were found in the female sperm storage organs. 3. Males have backward-pointing spines on the parts of their genitalia that reach the sperm storage organs. 4. Clumps of sperm were found on the male genitalia after the male withdrew from the female. Together, these results suggest that selection could be acting on the size and shape of male genitalia in Calopteryx. Subsequent studies suggest that similar processes occur in other odonate species.

More recently, investigations into the form and function of female reproductive structures have supported the cryptic female choice hypothesis for the diversification and elaboration of mating structures. Mechanisms have been found by which females could control the use of sperm (Eberhard 1996). This appears to be the case in the Caribbean fruit fly, *Anastrepha suspensa*. In this species, females have multiple spermathecae and store different amounts of sperm in each spermatheca (Fig. 4). Females have thin spermathecal ducts leading to the bursa copulatrix. Each of the spermathecae has a separate valve that could potentially be used by females to control the storage and release of sperm. These data suggest that female *A. suspensa* have the ability to discriminate between the sperm of different males by controlling the storage and release of the sperm. Whether they use this ability has not been established.

These studies of form and function of mating structures are important for understanding how selection might act on these structures, but they are not actual tests of the sexual selection hypothesis. To demonstrate sexual selection, one must show that differences in the mating structures result in differential access to gametes. Very few studies have actually tested this. In fact, in the insect literature, I am aware of only four studies that actually test for differential fertilization success based on differences in mating structures, although there are other studies that relate differences in mating structures to differences in access to mates (e.g., Arnqvist et al. 1997). I will review two recent studies that test for sexual selection on mating structures.

Case Study I: *Gerris lateralis*

The first case is a recent study by Arnqvist and Danielsson (1999) on the water strider, *Gerris lateralis*. They studied the effect of variation in reproductive and non-reproductive structures on

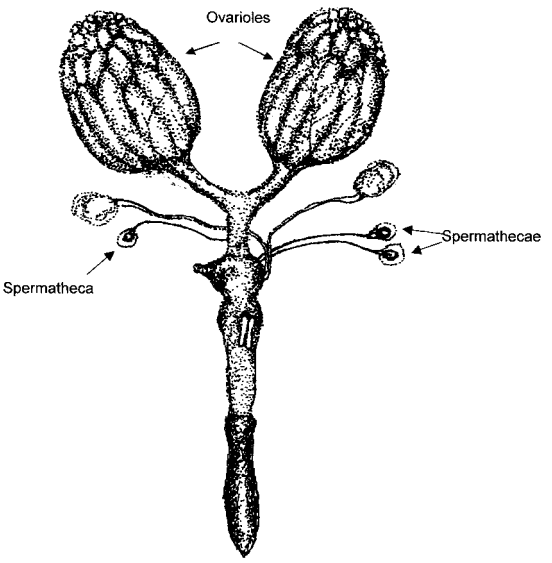

Fig. 4. Female reproductive structures of *Anastrepha suspensa*. Drawing by A. Fritz, printed with permission.

sperm precedence of the first and second males to mate with a female. There was evidence for sexual selection acting on sclerites that are found in the distal portion of the aedeagus. Although the function of these sclerites is not known, they appear to play a role in the placement of the aedeagus within the female reproductive tract and/or stimulation of the female.

Arnqvist and Danielsson (1999) found that the shape of the lateral sclerites of the first male to mate and the dorsal and ventral sclerites of the second male to mate affect sperm precedence. In addition, the degree of the effect of the ventral sclerite of the second male on sperm precedence depended on the size of the female. Together, these results suggest that selection acts on male mating structures in *G. lateralis* and that the strength of selection depends on the distribution of female phenotypes in the population. However, two questions remain unanswered about the selection process. First, it is unclear whether selection is acting directly or indirectly on the sclerites. It is possible that selection is actually acting on a trait that is correlated with the shape of the sclerites and not on the sclerites themselves. The authors controlled for many possible correlates, but, without manipulating the structures and randomly assigning males to treatment groups with differently shaped structures, it is difficult to infer causal relationships. Second, the mechanism by which selection is acting is also still unclear. It could be that (1) the shape of the sclerites allow males to position their own sperm or the sperm of other males in such a way that they have an advantage or (2) females use sperm of certain males preferentially depending on the shape of their sclerites.

Case Study 2: *Calopteryx haemorrhoidalis*

A study of damselfly reproduction provides more evidence of selection acting directly on a mating structure. This study is on a species of calopterygid damselflies, the same group in which Waage (1979) found sperm removal by males. Cordoba-Aguilar (1999) found patterns of sperm storage in *Calopteryx haemorrhoidalis* similar to those that Waage (1979) found in *C. maculata*, suggesting that sperm removal was also occurring in *C. haemorrhoidalis*. However, in *C. haemorrhoidalis*, the male genitalia could not get into the spermatheca, ruling out the possibility of direct sperm removal by males. Instead, Cordoba-Aguilar (1999) proposed that males stimulate females to eject sperm. Females have two sclerotized plates in their reproductive tract each bearing sensilla. When eggs pass by these plates, the plates are distorted and this distortion sends a stimulus through an abdominal ganglion to the sperm storage organs. The sperm storage organs respond by ejecting sperm for fertilization. During copulation, the male genitalia distort these plates in a manner similar to that of eggs passing through. Females with more sensilla store less sperm when their copulations are interrupted than females with fewer sensilla.

Cordoba-Aguilar predicted that males with wider genitalia would stimulate the sensilla more and stimulate the females to eject more sperm. He tested this prediction experimentally by simulating copulations using genitalia that he had removed from males. He used only the portion of the genitalia that normally makes contact with the plates to control for the effect of any correlated characters and to ensure that no sperm was removed directly by the male genitalia. Females mated with males with wider genitalia stored less sperm after simulated mating than females mated with males with narrower genitalia. However, the mechanism of sperm ejection is still poorly understood. It is very difficult to distinguish whether this is a case of female choice, male-male competition, or sexual conflict.

Connecting Intraspecific Selection with Interspecific Diversification

These two case studies are among the first to demonstrate sexual selection on mating structures. However, no studies have yet connected selection on mating structures within a species to diversification of mating structures between species. A group of grasshoppers found in Florida offers an excellent opportunity to study this connection (Fig. 5) These are the brachypterous (short-winged) species of the genus *Melanoplus* (Capinera et al. 1999; Deyrup 1996; Hubbell 1932, 1984; Squitier et al. 1998). In Florida, most of these species are found only in sandhill and scrub habitat. Because much of this habitat occurs in patches in Florida (Myers 1990; White 1970), some of the species are effectively isolated from other similar species (Fig. 6). This group is

Fig. 5. *Melanoplus ordwaye* pair in copula at Goldhead Branch State Park, FL.

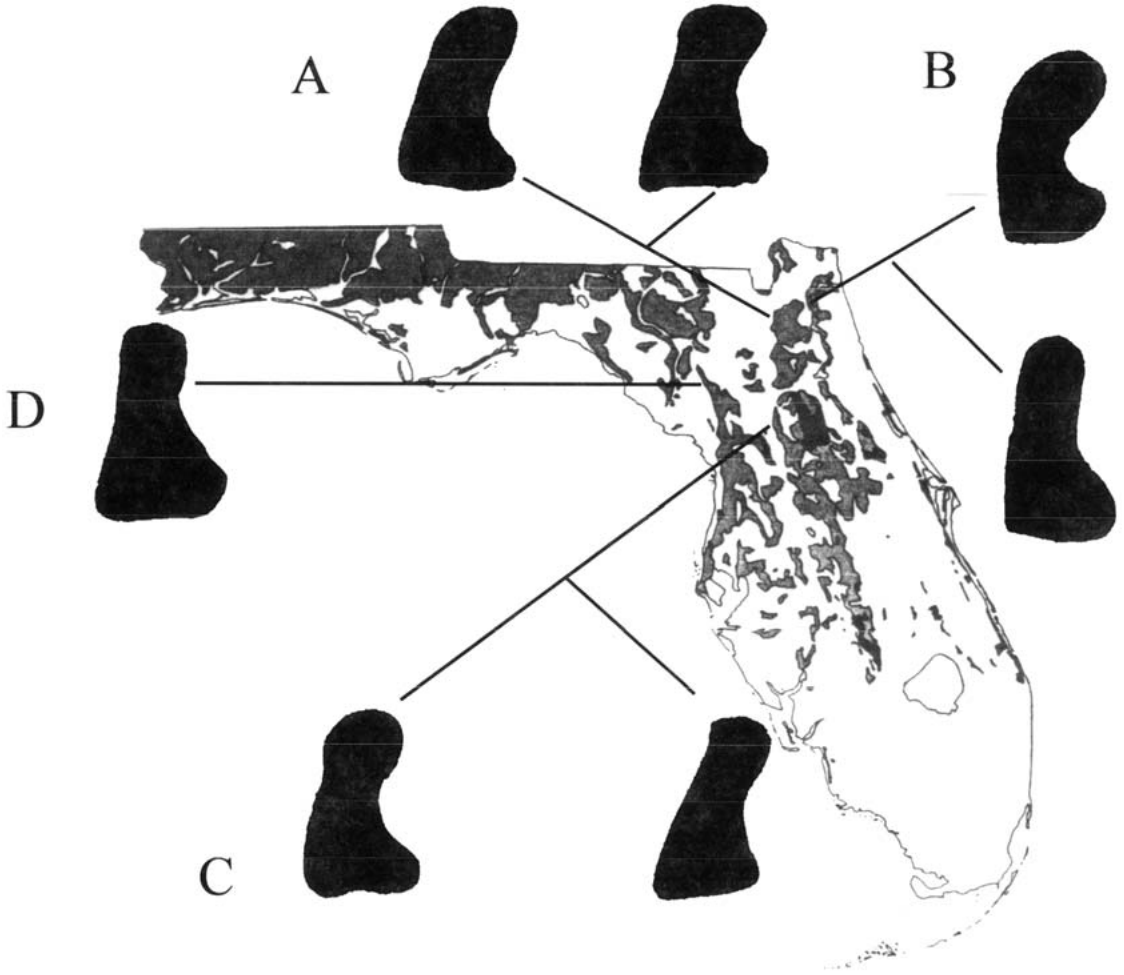

Fig. 6. Cerci of male *Melanoplus rotundipennis* from four sites in Florida. Map from Myers & Ewel 1990, reprinted courtesy of University Press of Florida. A. Goldhead Branch State Park; B. Welaka State Forest; C. Ocala National Forest; D. University of Florida's Thomas Farm (Gilchrist Co.).

characterized by extraordinary diversification of both internal and external male mating structures. For example, the cerci of different species form what Lloyd (1979) predicted as "a veritable Swiss Army Knife of gadgetry" (Fig. 7). The internal genitalia are similarly complex and diverse.

In addition to the interspecific variation in mating structures, there is also much intraspecific variation. For example, the cerci of *Melanoplus rotundipennis* vary both within and between populations. Figure 6 shows cerci from four populations of *M. rotundipennis*. The cerci differ both in curvature and in the width of the head relative to the rest of the cercus.

During copulation, the cerci appear to be used by males to gain access to the genital chambers of females (Fig. 8). The cerci squeeze against a flap that lies flat against the female's ventral surface, just below her ovipositor blades. This flap, called the egg guide, encloses the genital chamber, which is attached to the spermathecal tube. During coupling, the male's cerci appear to pinch either side of the egg guide (pers. obs.). Pressure on the sides of the egg guide results in the egg guide popping open, exposing the genital chamber. Sexual selection could act on the shape and size of the cerci through female choice in which females mate only with males whose cerci fit into the grooves of their egg guides (Eberhard 1998).

The shape of the cerci differ between populations of *M. rotundipennis* (Fig. 6). This variation suggests that selection could be acting differently in different populations. One could test this hypothesis in *M. rotundipennis* because it is possible to manipulate the shape and size of cerci (e.g., Krieger & Krieger-Loibl 1958), thus, removing the effect of correlated traits on reproductive success. It is possible to manipulate the shape and size of

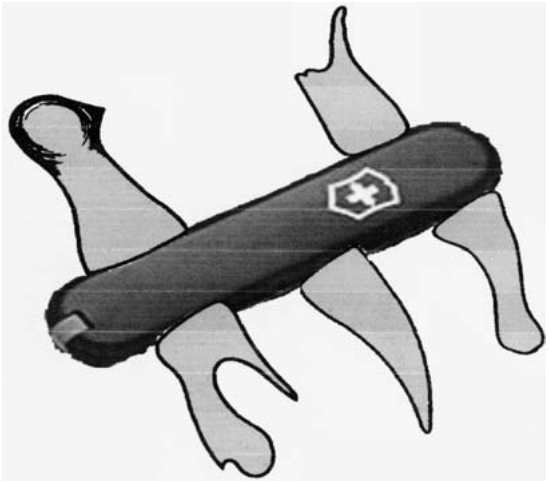

Fig. 7. "Veritable Swiss Army Knife" of cerci of different species of brachypterous grasshoppers of the genus *Melanoplus* found in Florida. Drawings of cerci from Capinera et al. 2001, with permission of author.

cerci by cutting them with microscissors. A similar method was used to test for sexual selection on male genitalia in the beetle, *Chelymorpha alternans* (Rodriguez 1995). In this species, males with longer genitalic structures (called "flagella") sire more offspring. This pattern could indicate direct selection on flagellum length or indirect selection on a correlated trait. Rodriguez distinguished between these possibilities by manipulating the length of males' flagella. Males with longer manipulated flagella sired more offspring, demonstrating direct selection on flagellum length. By using this method in *M. rotundipennis*, one could test whether and how cerci size or shape affected male reproductive success. Cerci size or shape could affect male reproductive success in a number of ways including increasing a male's sperm precedence or the female's oviposition rate or decreasing the likelihood that the female will remate (Eberhard 1996; Simmons 2001). Demonstration of sexual selection for different sized or shaped cerci in different populations would provide a connection between sexual selection on mating structures within a species and diversification of mating structures between species.

In conclusion, recent studies have established that sexual selection is acting on male mating structures. However, more work is needed in three main areas for us to have a better understanding of the evolution of mating structures through sexual selection. 1. We need to investigate and attempt to distinguish the processes by which sexual selection is acting on mating structures. As exemplified by Cordoba-Aguilar's (1999) research on *C. haemorrhoidalis*, it is often difficult to distinguish whether sexual selection on mating structures is a result of female choice, male competition, or intersexual conflict. More than one of these pro-

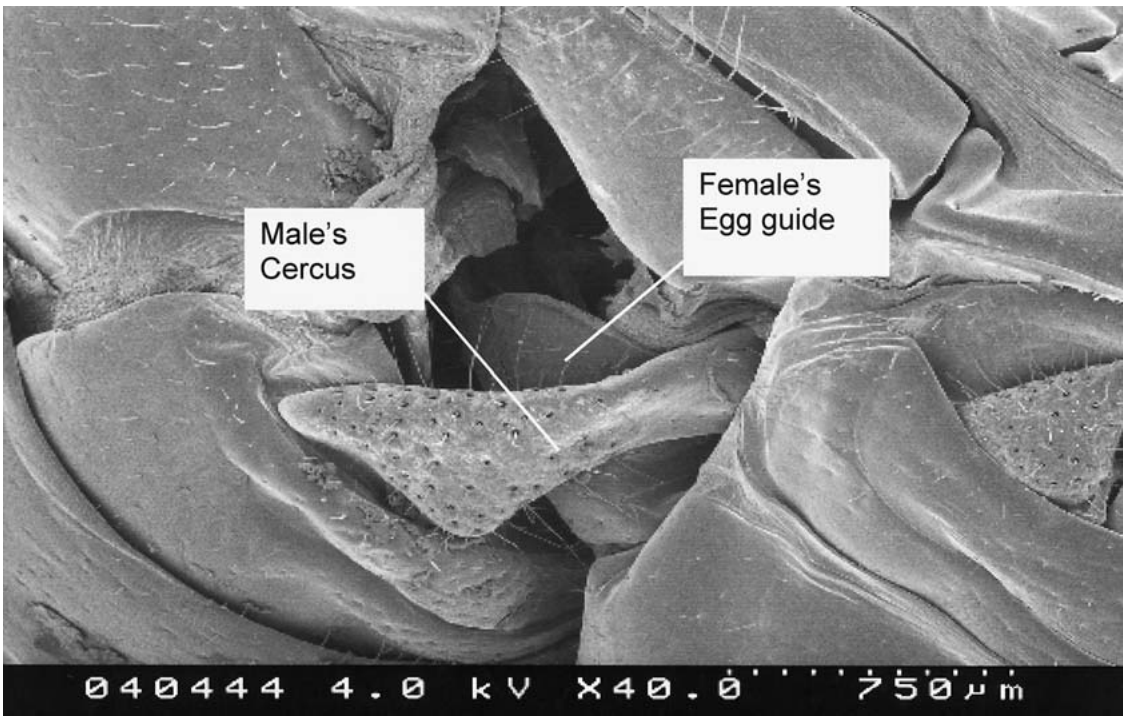

Fig. 8. SEM photo of external mating structures of *M. rotundipennis* pair in copula.

cesses could be acting simultaneously. We can understand sexual selection on mating structures more thoroughly by determining which of these processes are occurring. 2. We need to study the form and function of female mating structures and how selection acts on these structures. Female mating structures are a part of the selective environment in which male mating structures evolve, and vice versa. Understanding the biology of female mating structures will allow us to understand the sensory and physical environment in which male mating structures evolve. 3. We need to connect the process of intraspecific sexual selection on mating structures with interspecific diversification of mating structures. Current research on sexual selection on mating structures is focused predominantly on intraspecific processes. We must conduct studies across populations of the same species and closely related species to extrapolate how intraspecific sexual selection can result in interspecific diversification.

Acknowledgments

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ON WATER BEARS

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ABSTRACT

Tardigrades, or "water bears" are microscopic invertebrates that require water in their environment and are found in freshwater, marine and terrestrial habitats. The morphology and phylogeny of this "little known phylum" is described as are ways the naturalist might collect water bears. Examples of species distributions in different locations in the southeastern USA are given.

Key Words: tardigrade, taxonomy, phylogeny, ecology, collecting

RESUMEN

Los tardigrados, u "osos de agua" son invertebrados microscópicos que requieren agua en su ambiente y son encontrados en ambientes marinos de agua fresca y ambientes terrestres. Se describe la morfologia y la filogenia de este "filo poco conocido" y también las maneras que los naturalistas pueden recolectar los osos de agua. Se proveen ejemplares de la distribución de especies en lugares diferentes en el sureste de los Estados Unidos.

WATER BEARS: AT LAST, IN A PERSONAL REAL-LIFE INTRODUCTION

A line drawing in an already-classic entomology text book (Fig. 0) and a comment from a professor that "you are unlikely to ever see one" was my introduction to the idea of "tardigrades". Forty years later when visiting Jacksonville State University in northeastern Alabama a sign on a lab door said "Beware of the Bears", and was my invitation to actually see a living water bear. Frank Romano, the bear-room caretaker and proprietor—and now the Head of the Biology Department—said that bears were as near as the large tree on the lawn and that he could show me a living specimen in ten minutes!—not an idle boast, for in minutes and out of accumulated muck from the crotch of the tree he produced actual living water bears! For those who have never heard of tardigrades, nor the legend of their enigmatic and "by default" position in the Animal Kingdom, an "all about them and how to find them" encyclopedia entry may not mean much, but for some of us it is an invitation to a twilight zone where mythology becomes reality. (JEL)

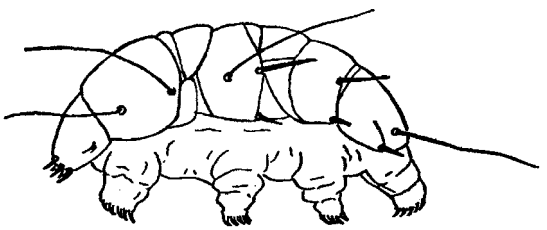


Fig. 0. The tardigrade line drawing in Herbert Ross's 1948 entomology text (p. 47), with the attribution "After U.S.D.A., B.E.P.A".

Tardigrades are microscopic invertebrates that belong to the Phylum Tardigrada (proposed by Ramazzotti in 1962). Active tardigrades require water in their environments and can be found in three main habitats; marine water, fresh water and terrestrial habitats (Kinchin 1994; Nelson 1991; Ramazzotti & Maucci, 1983). First described by Goeze in 1773. Commonly recognized as "Water Bears" (Wassar Bär) by observers, tardigrades are best classified as one of the "lesser-known phyla" (Nelson 1991). Tardigrades, the current name in use since the 18th century (adopted by Spallanzini in 1776) is also a descriptive name based on the animals lumbering gait (*tardi*-slow, *grade*-walker).

Tardigrades are generally considered cosmopolitan in their distribution and are commonly found in a variety of marine, freshwater, and terrestrial habitats: sand, algae, rooted aquatic vegetation, soil, leaf litter, mosses, lichens, and liverworts. These bilaterally symmetrical micrometazoans are generally flattened on their ventral side and convex on their dorsal side and average 250-500 µm in length as adults (see Dewel et al. 1993 for detailed morphology). Their body is composed of 5 somewhat indistinct body segments including a cephalic segment and four trunk segments each supporting a pair of legs that terminate in either claws and/or digits. The first 3 pairs of legs are directed ventrolaterally and are the primary means of locomotion, while the 4th pair is directed posteriorly and is used primarily for grasping the substrate (Fig. 1). Tardigrades feed by piercing the

cells of bacteria, algae, plants (mosses, liverworts, and lichens) or animals (protozoans, rotifers, nematodes, larvae, and other small invertebrates) with hardened stylets and sucking out their contents using their muscular pharynx (Fig. 2). In some cases, the whole organism is ingested. Detritus may also be a major nutrient source of some species. Regardless of their specific habitat (marine, freshwater, or terrestrial), all tardigrades are aquatic, since they require a film of water surrounding the body to be active. Some, those that are limno-terrestrial, can undergo cryptobiosis when environmental conditions become unfavorable (e.g., loss of the film of water) creating an environmentally resistant state. Thus, this latent state has a significant impact on the ecological role of limnoterrestrial tardigrades.

Despite their overall abundance and presumed cosmopolitan distribution (McInnes 1994), tardigrades have been relatively neglected by invertebrate zoologists. Because of the difficulty in collecting and culturing these organisms and their apparent lack of economic importance to humans, our knowledge of tardigrades has remained in a relatively nascent state since their discovery over 200 years ago (Nelson 1991).

Tardigrade Taxonomy

Marcus (1929) established the major taxa within the phylum Tardigrada splitting the group in two, forming the classes: Heterotardigrada (armoured tardigrades) and Eutardigrada (naked tardigrades). "Naked" and "armoured" refer to the cuticular dorsal plates found in terrestrial heterotardigrades, that are absent in eutardigrades. Morphological and anatomical differences are the only characters used to identify organisms to species. Within the heterotardigrades (armoured) the main features are cephalic appendages, cuticular extensions, claws and the pattern of dorsal cuticular plates (Fig. 3). Within the eutardigrade (naked) the more important morphological characteristics are the claws, the buccopharyngeal apparatus; and the cuticle structure (smooth, granulated or bearing tubercles) (Fig. 4).

Tardigrade taxonomy stems from a number of papers but primarily from Thulin (1928) who revised the systematics of the taxon, Marcus (1929) who wrote a chapter on tardigrades in "Classes and orders in the animal realm" (Vol. 5) and a book entitled "The animal realm" (1936), Ramazzotti (1962, 1972) who published monographs on the phylum tardigrada, and Ramazzotti and Maucci (1983) who collaborated to produce the monograph entitled "The phylum tardigrada" (English translation by Beasley 1993).

Marcus named the classes Eutardigrada (meaning 'true' tardigrades) and Heterotardigrada (meaning 'other' tardigrades). The genus *Macrobiotus* was described in 1834 (a eutardigrade) and the genus *Echiniscus* was described in 1840 (a heterotardigrade). A third class, Mesotardigrada (meso = middle), was established by Rahm 1937 for *Thermozodium esakii* discovered in a hot spring near Nagasaki, Japan. Neither type material nor type locality have survived and no other mesotardigrade have been discovered – a consensus from the last symposium on tardigrades (Eighth International Symposium on Tardigrada 2000) was that this should be removed from the classification.

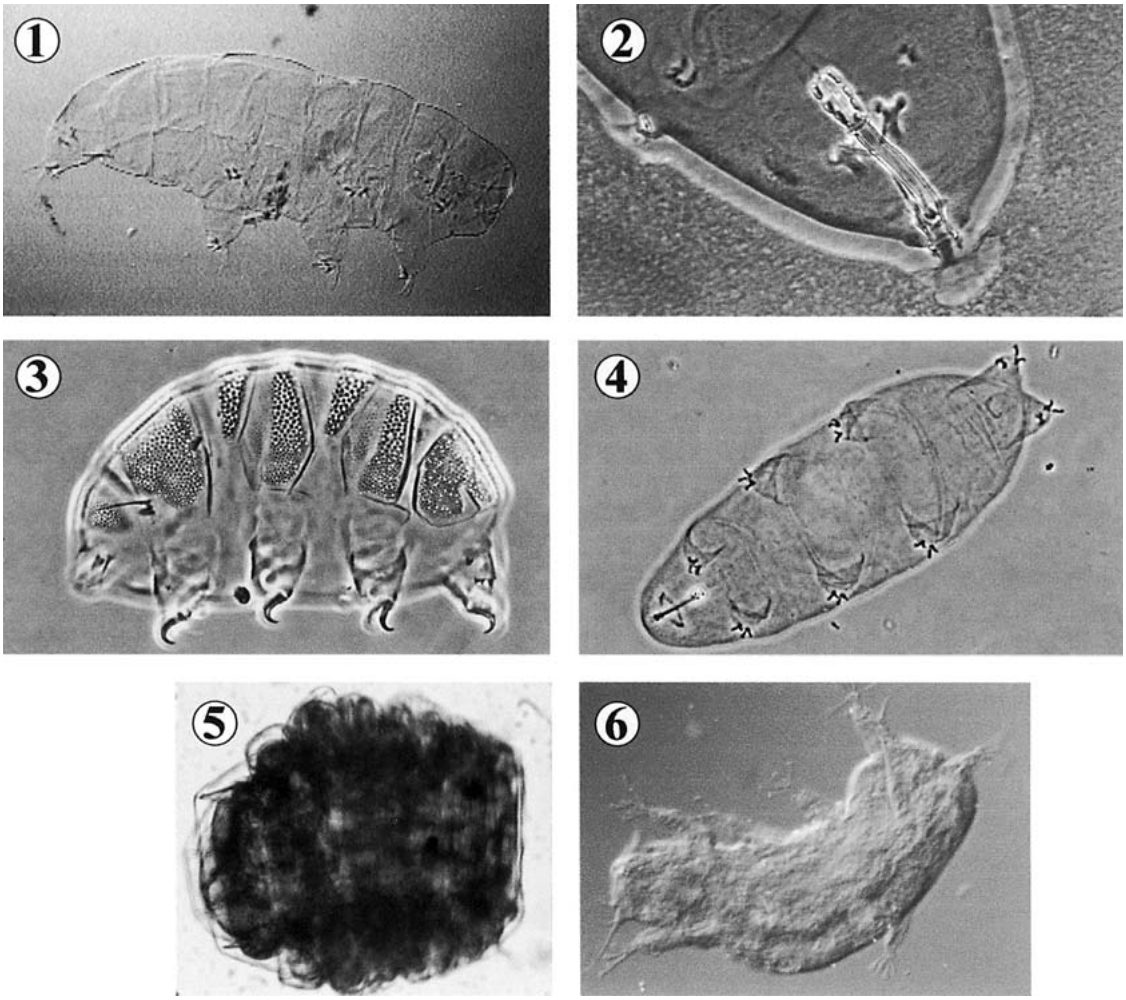
Tardigrade Phylogeny

Tardigrades have been closely aligned with arthropods and were described as primitive arthropods by Plate 1889 (from Kinchin 1994). The morphological characters that align them with arthropods are: vermiform animals with a cuticle, lobopodia (poorly articulated limbs) that terminate in claws, terminal mouths, caudal end (segment) terminating into the last pair of legs.

Ecology of Tardigrades

Active tardigrades require water in their environment and as noted can be found in three main habitats: marine, freshwater, and terrestrial habitats (Ramazzotti & Maucci 1983; Nelson 1991). Bryophytes, which hold water within the interstices of their cushions (mats), and leaf axils, provide ideal sites for terrestrial tardigrades. Species living in these wet terrestrial habitats are classified as limnoterrestrial, a useful term to distinguish the moss inhabiting species from the marine and freshwater species (Kinchin 1994). Ramazzotti and Maucci (1983) identified three common conditions that make the terrestrial habitat, such as mosses, suitable for tardigrades: (1) a structure that allows sufficient oxygen diffusion, (2) the ability to undergo alternate periods of wetting and drying, mainly through solar radiation and wind, and (3) one that contains sufficient food. In reference to moist-dry mosses, Kinchin (1994) stated that they share a drought tolerant pattern of adaptation to dehydration with animal groups, including tardigrades, named poikilohydry. This adaptation is advantageous to both the bryophyte and the bryofauna. Both the moss and the tardigrade can survive adverse conditions in a dormant state called a tun (Fig. 5) (anhydrobiosis for tardigrades).

Although Bertolani (1983) found that some tardigrade species were related to specific coastal dune mosses, other authors did not find enough evidence to support a direct correlation between particular moss species and particular tardigrade species (Nelson 1975; Kathman & Cross 1991). Hunter (1977) found no relationship between epiphyte species and species of tardigrades nor did Kathman and Cross (1991).



Figs. 1-6. 1) Whole mount of a Eutardigrade showing the indistinct segmentation and 4 pairs of legs. 2) Buccopharyngeal apparatus of a Eutardigrade showing the muscular pharynx, pharyngeal tube, stylet supports, and mouth. 3) Whole mount of a limnoterrestrial Heterotardigrade (*Echiniscus* sp.). 4) Whole mount of a limnoterrestrial Eutardigrade (*Hypsibius* sp.). 5) Tun formation in a Eutardigrade (*Milnesium tardigradum*). 6) Whole mount of a marine Heterotardigrade (*Batillipes* sp.).

In an effort to better understand when and where which tardigrades are abundant or rare, three ecological surveys (2 terrestrial and 1 marine) were conducted in Alabama, one on Dugger Mountain (Nichols et al. 2001), Alabama's second highest peak, one along Choccolocco Creek (Romano et al. 2001) within the riparian zone, and one on Dauphin Island. Five trees (*Quercus alba*) with cryptogams, three on north-facing slopes and two on south-facing slopes, were sampled seasonally at three sites (headwaters, midwaters, mouthwaters) along an unnamed tributary of the South Fork of Terrapin Creek. Trees were chosen based on their location outside the riparian zone at the peak, midpoint, or base of the north-facing and south-facing slopes along the creek. Seasonal and altitudinal variations in the distribution of the populations on the north- and south-facing slopes were determined. Significant seasonal and altitudinal differences were found in tardigrade abundance from samples collected at specific sites and between north- and south-facing slopes. Pooled data showed no differences in the overall abundance or number of species at each altitude. However, significant seasonal differences in both abundance and number of species were seen in pooled samples. Six sites along Choccolocco Creek were selected and 3 trees with mosses within each were surveyed for an 18 month period. No significant difference was found between tardigrade occurrence (total number of individuals) and season, moss genera, or tree species. However, there was a significant relationship be-

tween the number of tardigrades and site, indicating the need for additional replicate samples. A marine meiofauna survey of subtidal regions of Dauphin Island, AL in the northeast region of the Gulf of Mexico was initiated 1999. Samples were taken at mile intervals from the Mobile Bay side (north) and the Gulf of Mexico side (south). A sample consisted of 500 cc's of sand collected from the subtidal zone. Meiofauna were counted and tardigrades extracted from samples. A total of 20,846 meiofaunal organisms have been observed from 11 samples. Nematodes account for 69.1%, harpactocopepods account for 13.5%, and tardigrades account for 11.1% of the collection. Miscellaneous organisms make up the remainder of the collections (5.8%) containing organisms such as foraminiferans, bivalves, cnidarians, polycheates, and kinorhynchs. The genus *Batillipes* dominated the tardigrade collection (Fig. 6).

Tardigrade Collecting

The best source of tardigrades is within moss growing on the bark of live trees or leaf litter. Moss on rocks is okay but contains a lot of dirt, making the animals even more difficult to find. Moss on soil is even worse, although you will find tardigrades in about 50% of the samples. Moss on rotten logs has very few, if any tardigrades, and you might skip that habitat. Lichens on trees and rocks are sometimes fruitful.

Following the procedure of Nelson (1975) soak the moss sample in a stoppered funnel in tap water (a bucket for leaf litter) for at least 3 hours (3-24 hours). You can leave the samples overnight in water. Realize that you are trying to induce anoxybiosis so that the tardigrades release their hold of the moss plants and are more easily removed. Remove the moss and squeeze the remaining water out into a clean beaker or jar. Some samples require vigorous shaking and squeezing to remove a sufficient quantity of tardigrades. Let the water and collected materials in the jar settle and then decant the top water. Pour the bottom layer of water and debris into a collecting jar. If too much debris, especially dirt, has been collected, the material may be sieved through a nested series. Tardigrades, and eggs, will be trapped on a #325 (45 µm) screen. Be sure to collect material from 2-3 different sized sieves, since larger tardigrades may be trapped by these. Each piece of leaf litter should be rinsed and the water in the bucket poured into a nested sieve series and collected as above.

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ADAPTATIONS OF NEMATODES TO ENVIRONMENTAL EXTREMES

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ABSTRACT

Nematodes are a highly diverse group of organisms that show a variety of adaptations to extremes in soil and plant environments. Developmental dormancy and diapause are important for seasonal survival and long-term longevity of eggs in some species, whereas changing sex ratios may improve survival chances of the next generation in some instances. More direct and immediate responses to environmental conditions include aggregation or the formation of relatively resistant dauer larvae. Many nematodes can undergo temporary quiescence in response to environmental stress, and entry into anhydrobiosis or other extreme states allows long-term survival in unusually stressful environments. These inactive survival stages may make up a substantial proportion of the nematode population in some terrestrial environments.

Key Words: anhydrobiosis, dormancy, nematode survival, plant-parasitic nematodes, soil ecology

RESUMEN

Los nemátodos son un grupo de organismos sumamente diverso que demuestra una variedad de adaptaciones a los ambientes extremos de suelo y plantas. La latencia desarrollada y la diapausa son importantes para la sobrevivencia estacional y la larga longevidad de huevos en algunas especies, mientras que cambiando la proporción de sexos puede mejorar la probabilidad para sobrevivir de la proxima generación en algunos casos. Las respuestas más directas e inmediatas a la condiciones ambientales incluyen la agregación o la formación de larvas del estadio "dauer" (etapa alternativa adaptada para su supervivencia) relativamente resistentes. Muchos nemátodos pueden pasar por una quiescencia temporaria en respuesta al estres ambiental, y entrar a la anhidrobiosis u otros estados extremos permite la sobreviviencia de largo plazo en ambientes extraordinariamente severos. Estos estadios inactivos de sobrevivencia pueden representar una proporción substancial de la población de nemátodos en algunos ambientes terrestres.

Nematodes are a diverse group of invertebrates abundant as parasites or free-living forms in soil, freshwater, and marine environments. The more than 15,000 described species probably represent only a small portion of the total members in the Phylum Nematoda (Barker 1998). The soil is a particularly rich habitat for nematodes, with about 26% of described genera inhabiting soil as bacterivores, fungivores, omnivores, predators, or plant parasites (Wharton 1986). Added to this are soil-dwelling stages of parasites on insects or other animals, as well as freshwater genera that colonize soil to varying degrees. A moisture film is necessary for normal nematode activity (Wallace 1973), and therefore soil moisture, relative humidity, and related environmental factors directly affect nematode survival.

The soil environment offers varying degrees of protection for nematodes from dehydration. Parasites that are inside plant roots or insects enjoy optimal moisture and protection from desiccation as long as the health of the host persists. Life stages or species that do not live inside a host find protection in moist soil, but risk increasing exposure to dehydration as soils dry. Hazards increase as the soil-air interface is approached (Womersley 1987). A few unusual genera of plant parasites, such as *Anguina*, *Ditylenchus*, and *Aphelenchoides*, risk exposure in air as they climb (under humid conditions) to infect aerial plant parts. Risk may increase further as above-ground plant parts (leaves, seeds, etc.) dry up or die along with the nematode parasite inside. This overview introduces some of the strategies that soil-inhabiting nematodes use to cope with deteriorating environmental conditions and with particularly severe conditions.

ADAPTATIONS IN THE LIFE CYCLE

The most generalized life cycle of a nematode involves an egg, four juvenile stages (referred to as J1 to J4), and the adult. In many species, the appearance of juveniles and adults are similar, but great diversity exists in the life cycles of this large group (Wharton 1986). The life cycle of some nematodes offers built-in opportunities for resisting environmental stresses, such as a protective cyst that covers the eggs of some species. Many nematodes undergo the first molt in the egg, retaining the protection of the eggshell for the developing J2.

Developmental Dormancy and Diapause

Diapause and other delays in development that are common in insects (Chapman 1971; Romoser & Stoffolano 1998) occur in some nematodes as well (Evans & Perry 1976; Wharton 1986). Although diapause is not necessarily a result of adverse environmental conditions nor ended by favorable conditions (Chapman 1971; Evans & Perry 1976; Wharton 1986), it is nonetheless a critical survival mechanism during cold seasons and in the absence of a host. The stimulation of egg hatching in *Meloidogyne naasi* Franklin by chilling is a well-known example of diapause in a nematode (Van Gundy 1985). In some species of root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* spp., *Globodera* spp.) nematodes, a portion of the eggs hatch quickly while others hatch slowly over time (DeGuiran 1979; Zheng & Ferris 1991; Huang & Pereira 1994). The distribution of egg hatch over time may be quite complex. Zheng & Ferris (1991) recognized four types of dormancy in eggs of *Heterodera schachtii* Schmidt. Some eggs hatched rapidly in water, some required host-root diffusate for rapid hatch, while others hatched slowly in water or in host-root diffusate. Stimuli for hatching and ending of dormancy in various species include such factors as temperature (Van Gundy 1985) or the presence of host plant or root leachate (Huang & Pereira 1994; Sikora & Noel 1996). The quality of the latter depended on crop cultivar, phenology, and other factors (Sikora & Noel 1996). Interpretation of dormancy and diapause in nematode eggs is further complicated in that the induction of dormancy in cyst nematodes varies seasonally, and may be dependent on temperature or host phenology (Yen et al. 1995; Sikora & Noel 1996). Diapause and developmental dormancy seem to apply mostly to the egg stage and to juvenile stages within eggs, although instances of diapause in later juvenile stages or adults are known, mainly in a few animal-parasitic nematodes (Evans & Perry 1976).

Sex Ratios

Sex ratios are environmentally determined in many nematodes, including amphimictic species and those that are primarily parthenogenetic (Triantaphyllou 1973). The production of males has been especially well-studied in the root-knot nematodes. In this group, the nematode hatches from the egg as a mobile J2, which migrates through soil and into plant root tissue, where it establishes a permanent feeding site. Once the J2 begins to feed, it becomes immobile, increases its body size, and progresses through subsequent molts, developing into a female that can reproduce parthenogenetically. Males can be very rare in root-knot nematode populations, but in some instances may comprise more than 60% of the population (Papadopoulou & Triantaphyllou 1982). A variety of stresses may lead to increased production of males. These include nutritional deficiency or reduced photosynthesis in the host plant, age of the host plant, plant growth regulators or inhibitors, increased nematode population density, presence of plant pathogens, level of host plant resistance, and even temperature or irradiation (Bird 1971; Triantaphyllou 1973). If stress is imposed during development, second-stage juveniles developing as females can undergo sex reversal, producing intersexes or males (Triantaphyllou 1973; Papadopoulou & Triantaphyllou 1982). Aside from the obvious advantage of producing fertilized eggs with perhaps a better chance of surviving adverse conditions, increased male production in root-knot nematodes results in the production of a mobile form that can leave an area or plant under stress (Bird 1971).

Rapid Responses to Environmental Stress

Protective strategies built into the life cycles of nematodes help to ensure survival of the current or subsequent generation. Some physiological and behavioral responses allow nematodes to react more quickly to environmental stresses. For example, *Steinernema carpocapsae* (Weiser) Wouts, Mracek, Gerdin, & Bedding can cope with changing levels in soil O_2 by alternating between aerobic and anaerobic metabolism (Shih et al. 1996). Many species of nematodes will coil in response to drying (Bird & Bird 1991).

Dauer Larvae

Many nematodes form a temporary stage called a "dauer larva" in response to various types of environmental or nutritional stresses. Depending on the nematode species, dauer larvae can be formed in J2, J3, or J4 stages (Bird & Bird 1991). They undergo modifications in the cuticle structure to decrease permeability (Bird & Bird 1991), and some forms retain the cuticle from the previous molt as additional protection (Evans & Perry 1976). Dauer larvae are relatively inactive, but can react if stimulated, and revert to the normal juvenile stage if conditions improve. Desiccation, depletion of food supply, crowding, or deterioration of an insect host are factors that can stimulate formation of dauer larvae (Wharton 1986; Bird & Bird 1991; Womersley 1993). The formation of dauer larvae in *Caenorhabditis elegans* (Maupas) Dougherty as the food supply declines is mediated by pheromones (Huettel 1986).

The abilities of dauer larvae to resist environmental stress and to recover quickly to normal stages vary from species to species. The J4, or preadult, of *Ditylenchus dipsaci* (Kuhn) Filipjev as well as the J3 can control water loss to such an extent that both stages could be considered as forms of

dauer larvae (Bird & Bird 1991). The J3 of *S. carpocapsae* is a relatively resistant infective stage that may be exposed on vegetation or the soil surface as it actively searches for insect hosts (Poinar 1979).

Aggregation

Occasionally, individuals of some nematode species will mass together forming large aggregations. Probably the best known example is the accumulation of large numbers of *D. dipsaci* on the surface of stored flower bulbs (Christie 1959). The nematode clumps may be so large that they are actually visible to the naked eye as whitish masses referred to as "nema wool." The masses probably offer some protection against desiccation (Cooper et al. 1971), and nematodes in the masses may exhibit other low moisture adaptations such as coiling and anhydrobiosis. In contrast, swarming, which refers to large coordinated population movements of nematodes, is believed to function more in dispersal and migration than in moisture conservation (Croll 1970). Nematode aggregation is difficult to study since large masses of nematodes building up in laboratory culture may not be typical of those found in nature.

Responses to Extreme Environments

Quiescence refers to a dormant state in which metabolism and activity are slowed down in response to environmental stress. Unlike diapause, the dormant state ends when the environmental stress is relieved, and nematodes then return to normal activity. A variety of environmental stresses may trigger quiescent states (Table 1). In extreme cases of prolonged quiescence, the metabolic rate may fall below detectable levels and appear to cease. This extreme dormant condition is referred to as anabiosis (Wharton 1986) or alternatively as cryptobiosis (Cooper et al. 1971). The term "anhydrobiosis" is used most often to refer to quiescent and anabiotic states, probably because desiccation is the most frequent and most studied cause of quiescence. The degree of quiescence observed among nematodes varies along a continuum from mild quiescence to anabiosis, depending on the nematode species involved and even within the same species (Wharton 1986). Most nematodes can show quiescence at some point, but relatively fewer species are capable of anabiosis. Anabiosis is not restricted to nematodes, but is common in some other invertebrate groups such as rotifers and tardigrades (Barnes 1980).

Table 1. Terminology applied to quiescent states of nematodes.

Environmental stress	Quiescent state in response to stress[1]
Desiccation	Anhydrobiosis
Low temperature	Cryobiosis
Osmotic stress	Osmobiosis
Low oxygen	Anoxybiosis

[1]These terms used in response to specific environmental stresses. The terms quiescence (least extreme) and anabiosis (most extreme) refer to the intensity of the quiescent state. Cryptobiosis is a synonym for anabiosis.

Nematodes in anhydrobiosis (including extreme anabiosis) can survive under remarkably severe conditions (Table 2). *Filenchus polyhypnus* (Steiner & Albin) Meyl was revived from a dry herbarium specimen after 39 years (Steiner & Albin 1946). Important observations and insights into the unusual phenomenon of anhydrobiosis have been provided by several reviews (Cooper et al. 1971; Demeure & Freckman 1981; Wharton 1986; Womersley 1987; Barrett 1991). During entry into anhydrobiosis, a gradual water loss occurs over time, as water content falls from 75-80% in active nematodes to 2-5% in anhydrobiotic forms (Demeure & Freckman 1981). Survival is best if nematodes dry slowly; most species are killed if drying occurs too quickly (Barrett 1991; Demeure & Freckman 1981). Anhydrobiotic nematodes will rehydrate in water, but there is a lag time between immersion and their return to normal activity (Barrett 1991). The lag time is normally a few hours, but can vary from less than an hour to several days, increasing with the intensity of anhydrobiosis (Cooper et al. 1971; Wharton 1986; Barrett 1991). Recovery is improved if rehydration is slow, and if nematodes are exposed to high relative humidity before being immersed in water. Repeated cycles of drying and rehydration decrease viability (Barrett 1991).

The mechanisms responsible for anhydrobiosis are not well understood, but decreased cuticular permeability and the condensation or packing together of tissues and organelles are often observed, and in some species, increased levels of glycerol or trehalose are noted (Demeure & Freckman 1981; Wharton 1986; Womersley 1987; Barrett 1991). Coiling is a typical behavioral response observed in anhydrobiotic nematodes, and in most anabiotic forms since they enter anabiosis through anhydrobiosis. However, the behaviorial response seems to depend on the factor inducing anabiosis, since *Aphelenchus avenae* Bastian coils in response to drying but relaxes in a straight position in response to low O_2 (Cooper et al. 1971).

Many of the extreme examples of anhydrobiosis (Table 1) are foliar nematodes that venture above ground or bacterivorous and fungivorous nematodes from dry soils. But anhydrobiosis is probably common in many types of nematodes, including plant parasites living in soil (Womersley 1987), entomopathogenic nematodes (Womersley 1990), and possibly even freshwater forms inhabiting temporary ponds (Wharton 1986; Womers-

TABLE 2. EXAMPLES OF NEMATODE SURVIVAL AFTER LENGTHY TIME IN ANHYDROBIOSIS.

Nematode	Normal active habits	Anhydrobiosis conditions[1]	Time in anhydrobiosis	Reference
Anguina agrostis	Foliar plant parasite	Dried plant material	4 yr	Fielding 1951
A. tritici	Foliar plant parasite	Dried plant material	9-30 yr	Fielding 1951
Ditylenchus dipsaci	Foliar plant parasite	Dried plant material	16-23 yr	Fielding 1951
D. dipsaci	Foliar plant parasite	-80°C	5 yr	Cooper et al. 1971
Filenchus polyhypnus	Foliar in moss	Dried plant material	39 yr	Steiner & Albin 1946
Acrobeloides nanus	Bacterivore in soil	Dry soil	6.5 yr	Nicholas & Stewart 1989
Panagrolaimus sp.	Bacterivore in soil	Dry soil	8.7 yr	Aroian et al. 1993
Plectus sp.	Bacterivore in soil	-190°C	125 hr	Cooper et al. 1971
Plectus sp.	Bacterivore in soil	-270°C	8 hr	Cooper et al. 1971
Dorylaimus keilini	Freshwater nematode	Dry mud	10 yr	Cooper et al. 1971
Helicotylenchus dihystera	Plant parasite in soil	Dry soil	250 d	Aroian et al. 1993
Pratylenchus penetrans	Plant parasite in soil, roots	Dry soil	770 d	Townshend 1984

[1]Most at room temperature except as noted. Subzero exposures in laboratory, free of dry plant material or soil.

ley & Ching 1989). Plant-parasitic nematodes living in soil or roots, such as *Rotylenchulus reniformis* Linford & Oliveira or *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven, are able to undergo rather extreme states of anhydrobiosis, but in general are not considered as successful at this strategy (e.g., less extreme anhydrobiosis, shorter time in anhydrobiosis) as some of the more extreme examples such as *D. dipsaci* (Townshend 1984; Womersley & Ching 1989), and their long-term survival under anhydrobiosis is lower (Wharton 1986).

CONCLUSIONS

Varying degrees of quiescence, particularly anhydrobiosis, enable nematodes to survive a variety of extreme conditions, including desert soils (Freckman et al. 1977), Antarctic climates (Pickup & Rothery 1991), dry fallow soils without hosts (Womersley & Ching 1989), or dispersal in dry seed, plant debris, or dust (Barrett 1991). The phenomenon may be more common in nature than formerly thought, if we consider that many common soil nematodes may use this strategy to some extent (Womersley 1987). In the plant parasite *P. penetrans*, for example, 22-31% of the population was in an anhydrobiotic state in soils dried quickly, while 58-70% of the population was in anhydrobiosis in soils dried slowly (Townshend 1984). It is likely that substantial portions of a nematode population in soil may be overlooked, since commonly used methods for extracting nematodes from soil may miss anhydrobiotic forms (McSorley 1987), for which specialized extraction methods are required (Freckman et al. 1977). Extreme states of anhydrobiosis appear to be more common in nematodes in water-stressed environments such as drying, above-ground plant parts, but nematodes active at the soil-air interface are also vulnerable to desiccation and would benefit from such strategies (Womersley 1987). The fungivorous genus *Aphelenchoides* comprised 65-75% of the nematode fauna in pine litter in Florida (McSorley 1993), and the capability of *Aphelenchoides* spp. and the closely related *Aphelenchus* spp. for anhydrobiosis is well known (Demeure & Freckman 1981; Wharton 1986). The bacterivores and fungivores living in litter environments are relatively unstudied compared to economically important plant parasites. However, it is possible that anhydrobiosis is a common phenomenon and that a high proportion of the nematode population may be in an anhydrobiotic state in extreme environments such as those at the soil-air interface, litter, above ground, or in very cold or dry climates. Anhydrobiosis is fairly typical among Antarctic nematodes, for example (Pickup & Rothery 1991; Wharton & Barclay 1993). Our ability to investigate and understand nematode ecology in these environments will remain limited unless the anhydrobiotic portion of the community is considered. Studies of such marginal and stressful environments have and will continue to yield more information on anhydrobiosis and other nematode survival strategies.

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THE EVOLUTION OF OVIPOSITOR LENGTH IN THE PARASITIC HYMENOPTERA AND THE SEARCH FOR PREDICTABILITY IN BIOLOGICAL CONTROL

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ABSTRACT

Ovipositor lengths are thought to reflect the egg-laying and host-searching behaviors of parasitoids. For example, parasitoids that attack exposed foliage feeders often have short ovipositors compared to species that must penetrate a substrate to reach a host. However, the relationship between host accessibility and ovipositor length is not apparent in a guild of braconids that oviposits in the larvae of frugivorous Mexican tephritids. While the longest ovipositors are up to 5× longer than the shortest, all attack roughly the same stages of their shared hosts, often in the same fruits. Nor is there any evidence that the shorter ovipositors represent a saving of metabolic resources and energy that is redirected toward egg production or greater ability to move. It has been suggested that if the ovipositor length of an introduced parasitoid is substantially different from the ovipositors of species already present, then it is more likely to find an empty niche in its new environment, become established, and add to the control of its host. However, with the present lack of a simple explanation for the variety of ovipositor lengths within the Mexican guild it is not clear how predictive ovipositor length would be in this instance. Until the evolution and maintenance of the various lengths is better understood it may be more circumspect to practice fruit fly biological control through the conservation and augmentation of parasitoid species already present.

Key Words: Hymenoptera, Diptera, Ichnuemonoidea, Braconidae, Opiinae, Chalcidoidea, Tephritidae

RESUMEN

Se piensa que la longitud del ovipositor refleja el comportamiento de los parasitoides para ovipositar y buscar el hospedero. Por ejemplo, los parasitoides que atacan hospederos que estan expuestos sobre el follaje de que se alimentan a menudo tienen ovipositores cortos comparados con las especies que tienen que penetrar un sustrato para alcanzar al hospedero. Sin embargo, la relación entre la accesibilidad al hospedero y la longitud del ovipositor en un gremio de braconidos que oviposita en larvas de tefrítidos mexícanos fruteros no es evidente. Mientras que los ovipositores más largos son hasta 5 veces más largos que el más corto, todos atacan más o menos las mismas etapas del hospedero compartido, a menudo en la misma fruta. Tampoco hay evidencia que los ovipositores más cortos representan un ahorro de los recursos metabólicos y de energia que es redirijido hacia la producción de huevos o ha una mayor mobilidad. Se ha sugerido que si la longitud del ovipositor de un parasitoide introducido es significativamente diferente de los ovipositores de las especies ya presentes, luego es más probable encontrar en ese nuevo ambiento un nicho vacio, establecerse, y añadir para el control de su hospedero. No obstante, con la falta de una explicación sencilla para la variedad en la longitud de los ovipositores en el gremio mexícano, no es claro cuan predicible la longitud del ovipositor puede ser en este caso. Hasta que se entienda mejor la evolución y mantenimiento de las diferentes longitudes puede ser más prudente practicar el control biológico de la mosca de la fruta a travéz de la conservación y aumento de las especies de parasitoides ya presentes.

The extended-piercing ovipositor is perhaps the key innovation that led to the diversity and abundance of the parasitic Hymenoptera. It allows feats of carnivory that are difficult or even impossible for the other great parasitoid group, the Diptera, and underlies the evolution of the distinctive "wasp" morphology. The wasp-waist for instance, is a pivot that provides the flexibility needed to position the ovipositor/stinger at the most appropriate angle to reach the host or penetrate a cuticle (e.g., Quicke 1997).

While in essence a tube attached to a mobile "delivery system", it is an over simplification to imagine ovipositors as just biotic hypodermic needles (Quicke et al. 1999). They have external and internal structures that help steer them along

their course, serrations hardened with heavy metal-protein complexes, internal channels that deliver venoms, and microsculpturing to help move eggs along often considerable distances (Quicke et al. 1999; Vincent & King 1996). However, one of their seemingly simplest properties, their length, has a number of complex ecological and behavioral implications.

Even a passing familiarity with the parasitic Hymenoptera reveals the considerable variety of ovipositor lengths within the group. Why do these egg-laying tools exist in all these various lengths? The obvious answer is "to do their job by reaching their hosts", recognizing that hosts have different types of bodies and cuticles, and occur in a diversity of environments, surrounded by different depths and forms of materials, from unobstructed air to solid wood. Price (1972; LeRalec et al. 1996) accounted for the differences in ovipositor length among the parasitoids of the Swaine jack pine sawfly, *Neodiprion swainei* Middleton, by considering the tasks facing the different species. Some attack buried pupae and others oviposit in larvae exposed on leaf surfaces (Price 1972). Those that lay eggs in pupae have long ovipositors, designed to reach through leaf litter, while those that attack foliage-feeding larvae have short ovipositors just long enough to penetrate the host's integument.

But, will ovipositors be lengthened to deal with every contingency the wasp might face? Or, assuming there are tradeoffs to ever increasing size, will selection favor a length for every species that is just sufficient to undertake the typical piercing-depositing job it is likely to face? Might there be an optimal length, neither a "deluxe" nor "economy" model? And if there is an optimal length, are the only factors of any importance in its evolution the type of host being exploited and the environment where the host occurs? The answer to the last question seems to be no—at least not all the time or in any straightforward manner. Consider for example the braconids attacking Mexican fruit flies (López et al. 1999).

In the state of Veracruz 10 species of Hymenoptera attack tephritid flies of the genus *Anastrepha* (e.g., López et al. 1999). Among these parasitoids are a suite of native opiine braconids: *Utetes anastrephae* (Viereck), *Doryctobracon areolatus* (Szepligeti), *Doryctobracon crawfordi* (Viereck), and *Opius hirtus* (Fisher). An exotic opiine, *Diachasmimorpha longicaudata* (Ashmead) originally from the Indo-Philippine region, was established in the region over 30 years ago (Ovruski et al. 2000). All are solitary, endoparasitic koinobionts (parasitoids whose hosts continue to develop after being attacked) that oviposit only in frugivorous tephritids and complete development within the host's puparium.

These species, both native and exotic, are geographically widespread and attack a wide range of fruit flies in a diversity of fruits (López et al. 1999; Sivinski et al. 2000). It is not unusual for several to occur in any particular locale, or even for more than one species to emerge from flies infesting a single piece of fruit; e.g., *U. anastrephae* and *D. areolatus* are commonly found attacking *Anastrepha obliqua* (Macquart) in the same *Spondius mombin* L. fruits (Sivinski et al. 1997) and up to 5 species of parasitoids have been recovered from a single piece of fruit (Lopez et al. 1999). But while they have many similarities with respect to host range, distribution, and life histories, there are substantial differences in their ovipositor lengths (Fig. 1). These range from being less than the length of the abdomen in *U. anastrephae* to several times the abdominal length in *D. crawfordi*.

While these sympatric parasitoids share overlapping opportunities for oviposition, it appears they are not able to take equal advantage of the pool of hosts (Sivinski et al. 2001). *Anastrepha* larvae infest fruits over a large range of sizes, from little tropical "plums" weighing a few grams to commercial mangos more than half a kilo in weight (López et al. 1999). All the braconids attack flies in the smaller fruits, but only those with longer ovipositors are common in larger fruits (Fig. 1). How do the short-ovipositor species persist, and even flourish? Could there be a cost to having a long ovipositor, one so great that an insect with fewer options for oviposition, but investing in "cheaper" equipment, is still able to compete?

There are certainly problems inherent in having a very long ovipositor. Occasionally, species such as the Peruvian ichnuemonid *Dolichomitus hypermenses* Townes and the Japanese braconid *Euurobracon yakohamae* Dalla Torre carry prodigious external ovipositors, up to 8 times as long as their bodies (e.g., Townes 1975; Fig. 2). Some African Torymidae (or perhaps aberrant Pteromalidae) with ovipositors between 5 and 6 times their body lengths, e.g., *Ecdamura* sp. and *Eukoebelea* sp., are the likely record-holders among the chalcidoids (Compton & Nefdt 1988). However, these are rare exceptions to the rule, and few ovipositors exceed the more modest relative length of 1.3 times the body (Townes 1975). One reason is that the greatest force can be applied to the ovipositor when it is held perpendicular to the cuticle of a host or to the surface of the surrounding medium, and to accomplish this the abdominal tip must be held at least an "ovipositor-length" above the surface (van Achterberg 1986). Females wielding moderately long ovipositors often assume a head down/abdomen in the air/tip toe position to gain the greatest possible elevation. But even if the optimal position can be attained, too great a force on too-thin an ovipositor can cause it to bend (termed Euhler buckling), and prevent effective penetration (Vincent & King 1996; Quicke et al. 1999). All other things being equal the danger of this buckling is greater the longer the ovipositor.

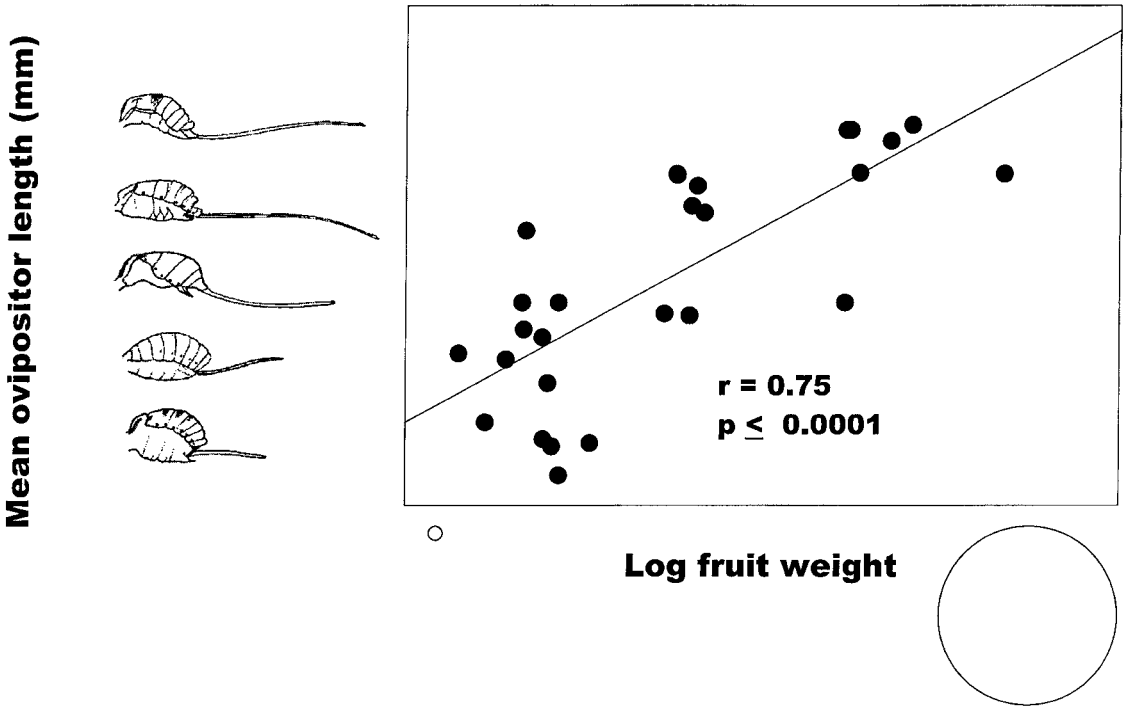

Fig. 1. The relationship between the mean size (weight) of a fruit sample containing tephritid larvae and the mean lengths of the ovipositors of the various parasitoids that attacked these particular larvae (see Sivinski et al. 2001). In general only parasitoids with longer ovipositors can exploit hosts in large fruits. The species, from top to bottom, are *Doryctobracon crawfordi*, *Diachasmimorpha longicaudata*, *Doryctobracon areolatus*, *Opius hirtus*, and *Utetes anastrephae*.

There are means of mitigating the positioning and buckling difficulties caused by extreme length. In *Megarhyssa* spp. ovipositors several times their owner's length can be effectively shortened by initially looping the shaft into a membranous sac at the tip of the abdomen (Townes 1975). The very long ovipositor of the parasitic orussid sawflies is coiled within the abdomen, and gripped by apodemes as it is extruded a bit at a time during drilling towards wood boring hosts. In this way the length of the exposed portion of the ovipositor is minimized, as is the problem of buckling (Cooper 1953; Quicke et al. 1999). In other instances, very long ovipositors are not used to penetrate tough substrates, but follow fissures or previously excavated tunnels through the medium surrounding the host. Under these circumstances, force and perpendicularity are not as critical and the ovipositor may meet the substrate at an angle of 120 degrees or less (van Achtenberg 1986).

In addition to exacerbating the penetration problems facing the ovipositor itself, increasing length can strain the "delivery system", the body of the wasp, by restricting movement, increasing wind resistance in flight, and making the insect more vulnerable to predators. Long ovipositors in a number of parasitoid taxa are held internally, e.g., that of the previously mentioned orussids is looped several times within the abdomen (Cooper 1953). All cynipoids and some chalcidoids carry the bulk of the ovipositor concealed in an internal pouch (Fergusson 1988; Quike et al. 1999). In chrysidids, platygasterids, and some scelionids the terminal abdominal segments telescope the ovipositor outward when in use and retract it when at rest (Kimsey 1992; Felid & Austin 1994). Even if not strictly internalized, the ovipositor is sometimes held out of the way by doubling its length back on the body. In the Vanhornidae it bends forward to rest in a groove on the ventral surface of the abdomen (Deyrup 1985). Leucospids carry the ovipositor curved over the dorsal surface of the abdomen, and in some platygasterids, such as *Inostemma*, the receptacle containing the internal portion of the ovipositor projects forward, "handle-like", from the base of the abdomen over the thorax (e.g., Goulet & Huber 1993).

No matter how useful it would be to have an ovipositor that could reach every host under the most difficult circumstances, it would seem that with all the problems, additional expenses and modifications that go along with size, the maximum length ovipositor may not be the optimal for

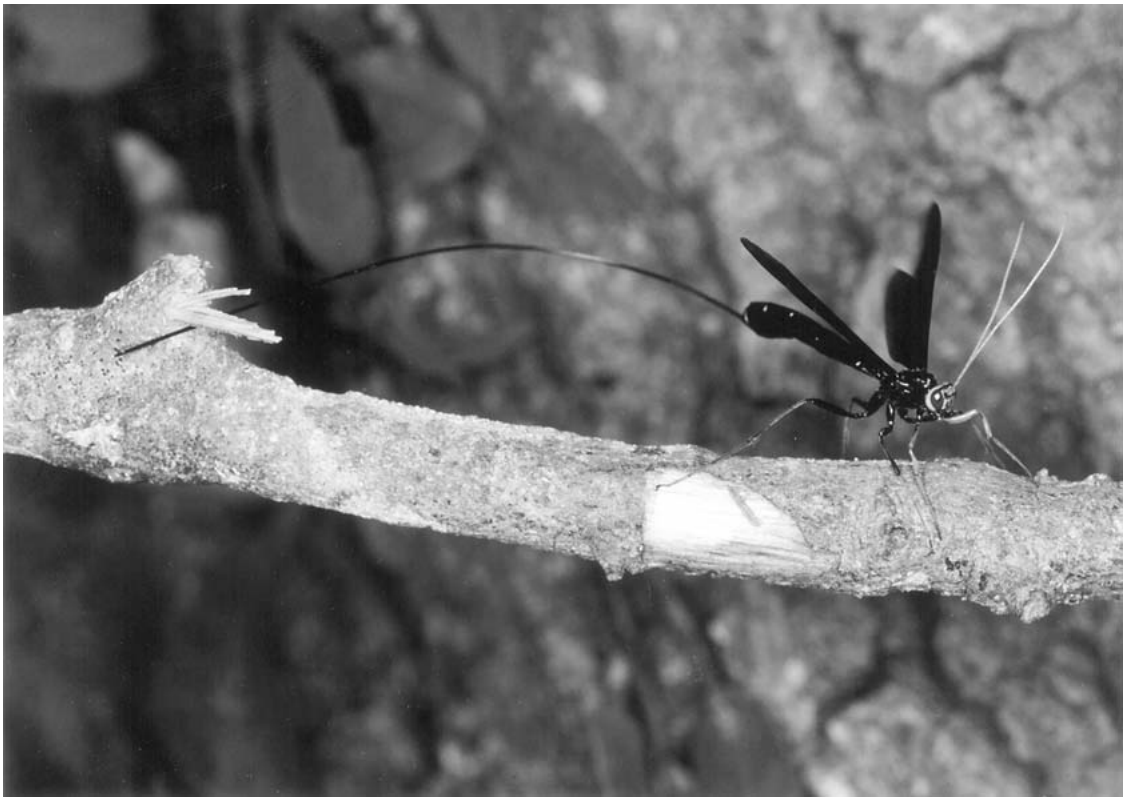

Fig. 2. A female *Megarhyssa atrata* (Fab.), a large ichnuemonid parasitoid with a very long ovipositor. The ovipositor can loop into a membranous pouch at the tip of the abdomen which shortens its exposed length. Such shortening prevents the ovipositor from buckling as it penetrates wood to reach the wasp's host.

the insect design as a whole. In terms of the Mexican braconids with the variety of ovipositor lengths, what might be the costs that prevent *D. crawfordi* (long) from displacing *D. areolatus* (medium) from displacing *U. anastrephae* (short)?

The energy and materials used to construct, maintain, and move an extended ovipositor could presumably have been spent elsewhere, perhaps in the production of more eggs, or in bigger flight muscles and better searching capacity. Of course, some fly larvae-hosts might be too deep in large fruits for the short-cheap ovipositor parasitoid to exploit, but access to these could be the benefit that makes it worthwhile for a competing species to continue to invest in a long-expensive ovipositor. That is, disruptive selection might result in a resource being shared by species with long and short ovipositors with few and many eggs, respectively.

The original prediction that fecundity should drop as ovipositors become longer, was made by Price (1973), who argued that if species with longer ovipositors deal with less accessible hosts, then, all other things being equal, handling time per oviposition should be greater and oviposition opportunities/unit of time should be fewer. In addition, since less accessible hosts are typically more mature, and because inevitable mortality occurs over the developmental period of the host, older, less accessible hosts should not be as abundant as younger, more accessible hosts. Both of the factors, longer handling time and fewer hosts, would contribute to lower potential rates of parasitism in species with long ovipositors. His hypothesis was supported by a strong negative correlation among species of Ichnuemonidae between ovipositor lengths and the numbers of ovarioles per ovary (which reflects the potential for egg production).

Is there a relationship between ovipositor length and fecundity in the Mexican braconids? No, there is not. The number of eggs does not significantly increase or decrease with ovipositor length. If there is a trend at all, it is in the opposite direction. The longer the ovipositor, the relatively more of the body is taken up by egg volume (No. of eggs*size of eggs) (Sivinski et al. 2001).

Though the "longer the ovipositor the lower the fecundity argument" is broadly supported when many species of Ichnuemonidae attacking a variety of host stages are considered, it is not as successful when looking at the one small guild of Braconidae attacking similar aged fruit flies un-

der what seem to be similar circumstances. But are circumstances really so comparable after all? Despite overlaps in host ranges, each species has one or more specialized foraging areas within its niche. If the fruits within these specialized areas differ in size or penetrability, then the hosts they contain differ in accessibility, and this difference in host accessibility might lead to differences in ovipositor length. Maybe ovipositor lengths have evolved in a variety of unrelated situations, and each length is so well suited to this core ecological "stronghold" that whatever advantage or disadvantage it faces with competing species exerts a relatively trifling selection pressure. For example, the short-ovipositored *O. hirtus* attacks the monophagous tephritid *Anastrepha cordata* Aldrich as it develops in *Tabernaemontana alba* Mill. (Hernandez-Ortiz et al. 1994). For unknown reasons it is the only parasitoid to commonly do so, and since the pulp of this fruit is relatively shallow there may be no selection for a longer ovipositor in this particular, and arguably important, tritrophic interaction. There are any number of other such "specializations" such as greater tolerance for heat or ability to flourish at high altitudes (Sivinski et al. 2000).

While the diversity of ovipositors can form engaging intellectual puzzles, their different lengths also have broad practical, agricultural implications. These arise from the argument by Price (1972) that ovipositor length might be a means of predicting which newly introduced parasitoids will be able to avoid competition within an already existing guild of natural enemies, and so have the best chance of successful establishment and the provision of additional control.

At this point, let us make a somewhat lengthy digression to discuss the history of prediction in biological control. Predictability is a supreme virtue in an applied science such as entomology where we strive to find some way of saying that this good thing will happen and this bad thing will not. The search for biological predictability has become an issue of increasing importance in terms of both invasive species that arrive in new locations by accident and potentially beneficial arthropods deliberately moved from one place to another. As the world becomes more homogeneous through the spread of weedy species, the aesthetic appreciation of biological diversity increases along with greater awareness of its economic and ethical implications (widespread similarity mitigates the evolutionary potential of life). There is a growing cultural mandate to prevent the accumulation of potential pests and extraneous biological control agents (Simberloff & Stiling 1996; Thomas & Willis 1998). The latter always present some risk, no matter how small, of attacking nontarget insects or plants. In cases where nontargets have relatively slow rates of increase, "apparent competition", where an organism harbors a natural enemy that also attacks a more vulnerable species and as a result becomes a superior competitor, can be potentially devastating (Bonsall & Hassell 1997; Hudson & Greenman 1998). Even something that is initially safe may have the capacity to adapt to a more diverse environment and increase its host range (Willamson 1996).

A means of judging the present predictability of biological control is to compare the rates of establishment and resulting abundances of deliberately introduced natural enemies with the fates of "invasive" organisms that arrive in new areas largely by chance. It seems that establishing a beachhead is a long shot for an invading organism, and can be described by the "Rule of 10s". Willamson (1996) estimated that only 1 accidentally introduced animal or plant in 10 becomes established and only 1 out of 10 of these becomes abundant and pestiferous. Interestingly, the odds of a deliberately introduced biological control organism becoming common enough to exert an economic impact are only somewhat better, perhaps 3 in 10 become established and 3 of those effect control (depending on how success is measured). Apparently, there is often a far from complete understanding of the relevant ecology of natural enemies and their prey, and hence a long standing interest in why some natural enemies "work" and others do not.

Among practitioners of biological control there have been several attempts to collect and synthesize the attributes of successful natural enemies in order to focus explorations and make establishments more effective and environmentally safe. Propagule pressure, the size of the released cohort, is important to the outcome of natural enemy establishment. In a survey of Canadian programs, increases in the numbers of released insects, from <5000 to >30,000, improved success rates from 9% to 79% (Beirne 1975; Willamson 1996). If fewer than 800 individuals were included in individual releases success occurred 15% of the time compared to 65% if more than 800 insects were involved, and more than 10 releases gave 70% success compared to 10% for programs using fewer releases. When Goeden (1983) examined the insects introduced for weed control he found long attack season, gregarious feeding, and ease of colonization to be the most important contributors to success. The last of these has implications for propagule pressure.

In addition to how the craft of biological control is practiced there are some ecological generalizations concerning the vulnerability of insects to their natural enemies that might result in more predictable control. For example, biocontrol has tended to be more efficacious when applied against specialist herbivores rather than generalists and against exposed rather than concealed feeders (Gross 1991). Hosts that suffer high maximum parasitism rates, and by implication have

fewer or less effective refuges to shelter within, are more likely to be successfully controlled (Hawkins & Gross 1992; Hawkins 1994). Within particular host taxa there are a number of even more specific correlations between vulnerability and type of natural enemy, and these relationships could be used to direct future establishment attempts. For example, Dyer and Gentry (1999) have examined the categories of predators and parasitoids that typically inflict high or low mortalities on Lepidoptera larvae with different morphological characteristics and defensive behaviors. Brightly colored larvae were likely to be rejected by wasps and bugs, but were attacked by ants and parasitoids, generalists were more likely to succumb to predation than to parasitoids, while hairy species were relatively immune to ants and bugs but fell victim to wasps and parasitoids, and so on. On the basis of their analysis they suggest that the generalist feeding habits of the infamous pest caterpillars of the genus *Spodoptera* (Noctuidae) are the reason they have not been successfully controlled by parasitoids, despite considerable efforts, and argue that in the future, predators, such as carabid beetles, might be more profitably employed.

In addition to morphology and ecology, the history of a pest and parasitoid interaction might be used to predict successful biological control. Hokkanen and Pimentel (1984) proposed that new associations between insects and natural enemies resulted in substantially greater mortality and a higher degree of pest suppression. The basis of their thesis was that long standing interactions will tend to be more benign since a prey species will have had ample opportunities to adapt to its hunter(s), but that it will be relatively defenseless when confronted with a novel set of weapons and hunting tactics. There are at least two criticisms of this theory. One is that the data used to substantiate the greater vulnerability of prey to new parasitoids can be reinterpreted to reach the opposite conclusion (Waage 1991). The second is that there is accumulating evidence that long term associations are typically more virulent than new ones: i.e., it is the natural enemies, including pathogens and parasites, that are ahead in arms races with their victims, and that familiarity has resulted in increasingly effective weapons and hunting tactics (e.g., Herre 1993; Ebert 1994; Kraaijeveld et al. 1998). While the opposite of earlier thinking, this emerging generalization of familiarity breeding lethality can be used as a predictive tool. It suggests that the closest possible match between the original populations of exotic pests and the populations of natural enemies that attacked them would tend to be most efficacious. However, as noted by Waage (1991), there seem to numerous exceptions to this rule of thumb, and in a practical sense one should not ignore any potential natural enemy regardless of origin.

There are also population characteristics, i.e., the distribution and abundance of a parasitoid in nature that might predict usefulness in a biological control program. Rare species on the periphery of host populations may be less competitive than other natural enemies, but be better foragers at low host densities. Such a species might do very well indeed if it could be introduced by itself into a new environment to deal with an exotic pest (e.g., Force 1974).

Now let us return to Price (1972) who reasoned that ovipositor length could be yet another means of estimating the likelihood that an exotic species would become established and whether it would disrupt the composition of an already existing native guild. He followed Hutchinson (1959) and Schoener (1965) who found that a "trophic apparatus", such as a bird's beak or an ovipositor, typically differs in size among sympatric species at the same trophic level, and that these differences in size are related to the differences in foraging behaviors that allow the species to coexist. A ratio of the larger to the smaller apparatus of 1.15 indicates sufficient niche separation in terms of the resource the apparatus is used to exploit. When ovipositor length ratios were examined in the guild of parasitoids attacking the Swaine jack pine sawfly, Price found that this threshold ratio was exceeded in comparisons among native species, but that the introduction of a European exotic had created a too close pairing of lengths between itself and a native species, and that there was already evidence of competitive displacement.

In the spirit of Price's search for predictability through ovipositor length, what do the various ovipositors of the Mexican braconids reveal about the potential for expansion through new introductions of this fruit fly parasitoid guild where it is already established, and about the use of its constituent species in future tephritid biological control programs elsewhere? There is the well-established relationship between ovipositor length and the size of the fruit a parasitoid can effectively forage upon. One might prefer to introduce a long ovipositored species such as *D. crawfordi* into new habitats dominated by large fruits. Other than this, there is little that can be said with certainty. There are obvious differences in ovipositor lengths, much as there are in Price's sawfly parasitoids. But, while the sawfly-parasitoid ovipositors are clearly due to distinct differences in foraging for different host stages, the same cannot be easily said for the Mexican tephritid-parasitoids. At this point it is difficult to say with any conviction how the various parasitoids manage to coexist in sympatry, and what role the differences in their ovipositors play in their coexistence.

If attempts were made to improve fruit fly biological control in Mexico are there "empty" niche spaces where exotic parasitoids would fit? Given our lack of understanding how the present diver-

sity of tephritid parasitoids is maintained this is a troubling question to address. There is some circumstantial evidence of displacement of the long-ovipositored native *D. crawfordi* by the long-ovipositored exotic *D. longicaudata* (Sivinski et al. 1997), but the way in which this may have occurred remains obscure.

What is the best response to ignorance of the consequences of projected parasitoid introductions? More study is an obvious answer, but what if the sort of study that could predict success or dangerous failure requires time, and that during that period of study inactivity is impractical? We suggest that one way to deal with the present confusion and to best adhere with the applied-biology dictum of "do no harm" is to fully exploit what is already there; i.e., to conserve the existing guild and enhance its effectiveness through habitat manipulation.

For example, only 3.5% of the >200 species of *Anastrepha* are of any economic importance, yet a number of benign, generally monophagous, species developing in native fruits harbor the same parasitoids that attack notorious pests such as the West Indian fruit fly, *A. obliqua*, or Mexican fruit fly, *A. ludens* (Loew) (Aluja 1999). By encouraging the replanting of these sometimes endangered fruit trees in the vicinity of orchards it may be possible to support large numbers of parasitoids that will suppress pests that threaten crops destined for local consumption or markets (Aluja 1999). In addition to insect control and the conservation of disappearing plants and the flies and other arthropods associated with them, replanted fruit trees can be managed as timber and harvested for a profit. *Tapirira mexicana* Marchand, a tree that supports *A. obliqua* but also large numbers of 4 species of braconid parasitoids, has a wood equal in quality to mahogany (Terrazas & Wendt 1995).

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THE AFRICAN CLUSTER BUG, *AGONOSCELIS PUBERULA* (HETEROPTERA: PENTATOMIDAE), ESTABLISHED IN THE NEW WORLD

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ABSTRACT

An African species of Pentatomidae, *Agonoscelis puberula* Stål, is reported for the first time from Mexico, the southern United States and the islands of Jamaica and Hispaniola, where it has now established. The oldest Western Hemisphere record dates from 1985. This species has gone unrecognized probably because of its close resemblance to species of the New World genus *Trichopepla* Stål. The primary host plant of *A. puberula* is the introduced weed, common horehound, *Marrubium vulgare* L. It has also been reported damaging winter fruits in South Africa.

Key Words: cluster bug, horehound, stink bug, invasive species

RESUMEN

Una especie Africana de Pentatomini, *Agonoscelis puberula* Stål, es reportada por primera vez para México, sur de Estados Unidos y las islas de Jamaica y Española, en donde se ha establecido. Los registros en el hemisferio oeste más antiguos son de 1985. Esta especie no había sido reconocida probablemente por su gran parecido a las especies del género del Nuevo Mundo *Trichopepla* Stål. La planta hospedera primaria de *A. puberula* es la hierba conocida como marrubio, *Marrubium vulgare* L. También ha sido reportada dañando frutos de invierno en Sudáfrica.

Translation provided by author.

In this paper we give the first report of the African pentatomid bug, *Agonoscelis puberula* Stål, in the New World. Established populations of this stink bug have been discovered in the United States, Mexico, and the islands of Jamaica and Hispaniola. The species was first found among a series of specimens collected in 1991 near the town of Yautla in the state of Morelos, Mexico. These specimens were tentatively identified by one of us (GOL) as an undescribed species of the north-temperate genus *Trichopepla* Stål, to which they keyed in Rolston and McDonald's (1984) treatment of Western Hemisphere Pentatomini. Its discovery in the Greater Antilles led another of us (JEE) to recognize this stink bug as an introduced species of the genus *Agonoscelis* Spinola, one referred to in the economic literature as a "cluster bug" (Haines 1935).

Taxonomy and Recognition

As in *Trichopepla*, species of *Agonoscelis* are generally yellowish, often with a red tinge and with black punctures arranged in a pattern of irregular dark stripes, and a distinctly hirsute dorsum (Fig. 1). Other shared characteristics include the scent gland orifice attended by a short auricle; the post-frenal scutellum more than half the width of the scutellar base, and base of the abdomen lacking a tubercle. In both genera the head is elongate compared with other pentatomines. Our specimens range from 8-10 mm in body length. In spite of their similarity, *Trichopepla* and *Agonoscelis* have been split into different tribes. This anomaly arises largely because there is no consensus classification for the Pentatominae. American workers, such as Rolston & McDonald (1984), follow the tribal arrangement in Kirkaldy's Catalogue (1909) which places both genera in the Pentatomini. Asian workers, such as Ahmad et al. (1974), follow the arrangement of Distant (1921) which places *Agonoscelis* in the Eurydemini (=Strachiini). African workers, such as Cachan (1952), include *Agonoscelis* with the tribe Carpocorini. Inasmuch as there are no external morphological characters to distinguish *Agonoscelis* from *Trichopepla*, their placement in different tribes is problematic. According to McDonald (1966), the female spermatheca of *Trichopepla* is unique in lacking a sclerotized supporting rod

Fig. 1. *Agonoscelis puberula*, specimen from Morelos, Mexico.

and pumping region that is present in all other Pentatomines, including *Agonoscelis* (illustrated by Gross 1976). Such being the case, the separation of the two genera can be sustained, although the tribal-level separation seems dubious. *Agonoscelis puberula* has a distinctively marked hemelytral membrane featuring dark radiating stripes. This character is variable among species of *Agonoscelis*, but the membrane is unmarked in all species of *Trichopepla* (McDonald 1976); thus, the striped membrane allows quick recognition of this adventive stink bug.

The genus *Agonoscelis* has not been revised, although regional faunal treatments (Horváth 1904; Jensen-Haarup 1920; Cachan 1952; Yang 1962; Ahmad et al. 1974; Linnavuori 1975, 1982; Hsaio et al. 1977) provide means to diagnose many of the species. There are 22 nominal species of *Agonoscelis* including those of uncertain validity. Among the determined specimens available to us for study, representing six species, we noted that the male genitalia are distinctive to a given species, and it is on these characters that our determination relies. The New World invader is conspecific with specimens from South Africa identified by D. A. Rider and others as *A. puberula* Stål. The male genitalia of this particular species are illustrated by Linnavuori (1975) whose material was compared to Stål's types in Stockholm. We have deposited voucher specimens in the United States National Museum, the Canadian National Collection, the Instituto de Biologia of the Universidad Nacional Autonoma de México, at Texas A&M University, in the Florida State Collection of Arthropods, and in the collections of the authors.

Distribution

Agonoscelis puberula is native to southern and eastern Africa extending northward to the Arabian peninsula (Linnavuori 1982). Our oldest New World record dates from 1985 on the island of Jamaica. The first records for the United States are from Arizona in 1990. U.S. records include Arizona, New Mexico, and Texas. It is also well established in Mexico with records from Yucatan in the south to Nuevo Leon in the north covering the years 1988 to 2001. Our collection data includes the following specific localities and dates:

JAMAICA: St. Andrew Parish, 2 mi. S. Newcastle, 2-VIII-1985, C.B. & H.V. Weems Jr. & G.B. Edwards.

DOMINICAN REPUBLIC: La Vega: 21 Km S Jarabacoa, 18-26-VI-1994, C. & K. Messenger.

MEXICO: Guanajuato: San Miguel de Allende, 7-11-VIII-1988, G. B. Edwards; Chipicuaro, Presa Solis, 12-III-1997, E. Barrera & H. Brailovsky; Ojo Seco, 12-III-1997, E. Barrera & H. Brailovsky; San Antonio Emenguaro, 12-III-1997, H. Brailovsky, E. Barrera & G. Ortega-Leon. Morelos: Yautla, 3-V-1991, H. Brailovsky & E. Barrera. Distrito Federal: Piramides de Cuicuilco, 2-IV-1992, E. Gonzalez; Delegacion Iztapalapa, 1-VIII-1999, J. Contreras; Colonia Irrigacion, 18-VI-2001, H. Brailovsky. Mexico: Ixtapan de la Sal, 4-X-2000, H. Brailovsky & E. Barrera; Malinalco, VII-1996, E. Barrera. Guerrero: Tuxpan, 25-X-2001, H. Brailovsky, E. Barrera & G. Ortega-Leon. Hidalgo: Huichapan, 5-VI-1999, H. Brailovsky & E. Barrera; Huasca, 4-VIII-1995, H. Brailovsky. Michoacan: San Lorenzo, 24-X-2001, H. Brailovsky & E. Barrera. Oaxaca: Dominguillo, 18-II-1998, H. Brailovsky, E. Barrera & G. Ortega-Leon; Tehuacan-Oaxaca Km 140, 11-III-2000, H. Brailovsky & E. Barrera. Puebla: Tecamachalco-Tehuacan Km 1, 12-VI-1993, H. Brailovsky & E. Barrera; La Trinidad, 3-II-1994, E. Barrera & G. Ortega-Leon; La Trinidad, 13-II-1994, E. Barrera & G. Ortega-Leon; La Trinidad, 21-III-1994, E. Barrera & G. Ortega-Leon; Atlixco 23-IV-1994, E. Barrera & G. Ortega-Leon; Atlixco-La Trinidad, 29-V-1994, H. Brailovsky & E. Barrera; La Trinidad, 15-VI-1994, E. Barrera & G. Ortega-Leon; 5 Km SE Atlixco, 23-IV-1994, 15-VI-1994, H. Brailovsky, E. Barrera & G. Or-

tega-Leon; 2 Km W. La Trinidad, 19-III- 1994, G. Ortega-Leon & E. Barrera; Atexcal, 11-III-1994, E. Barrera & G. Ortega-Leon; Nicolas Bravo, 20-III-1993, H. Brailovsky, E. Barrera & G. Ortega-Leon; Tecamachalco, 6-I-1993, 27-I-1993, 12-VI-1992, 20-VII-1992, H. Brailovsky & E. Barrera; Atlixco, 18-VIII-1996, H. Brailovsky, E. Barrera & G. Ortega-Leon; Portezuelo, 10-II-1995, E. Barrera & G. Ortega-Leon. Queretaro: Pinal de Amoles, 27-IV-1998, 1-III-1998, E. Barrera & G. Ortega-Leon. Yucatan: Temax, 24-V-1995, E. Barrera & H. Brailovsky. Nuevo Leon: El Pinito, 3-IX-1995, D.B. Thomas & J. Burne.

UNITED STATES: Arizona: Pinal Co., Peppersauce Canyon, Santa Catalina Mtns., 9-IV-1991, C. Olson; Santa Cruz Co., Madera Canyon, 16-IV-1990, 17-VII-1990, 27-VII-1990, W. Jones; Patagonia, 8-VII-1994, B. Brown & E. Wilk; Santa Rita Mtns., Florida Canyon, 1-VIII-1992, W. Jones; Arrivaca Springs 1-VIII-1992, W. Jones. New Mexico: Hidalgo Co., 11 mi. NE Lordsburg, 31-VIII-2000, J. Huether. Texas: Concho Co., Eden, 29-XII-1999 [no collector].

Host Plants

At four separate Arizona localities a total of 26 adults was collected by one of us (WJ) on the pandemic weed, common horehound, *Marrubium vulgare* L. (Labiatae). At one of these sites nymphs were also present. This is also a known host plant for the Australian horehound bug, *Agonoscelis rutila* (F.) (Gross 1976). In South Africa, Haines (1935) reported that *A. puberula* breeds on its natural host plant in the summer, but overwinters in buildings and on fruit trees, sometimes clustering on the fruits and causing "considerable damage." Unfortunately, Haines neglected to state the species of the host plant or the fruit damaged in South Africa. Our specimens from Concho County, Texas, were found on December 29 among stems and leaves of live oak, suggesting that they were overwintering in this habitat.

Our colleague Thomas J. Henry (USDA-ARS) informs us that he has frequently identified *Agonoscelis versicolor* (F.), intercepted on cut flowers shipped to the United States from South Africa via the Netherlands. This suggests a plausible route for the entry of *A. puberula* which may have established because of the ready availability of an acceptable host plant. According to Correll and Johnston (1970), common horehound is widely distributed in North America, flowers throughout the year, and is a weed typical of waste places and roadsides.

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FEEDING AND SURVIVAL OF CITRUS SHARPSHOOTERS (HEMIPTERA: CICADELLIDAE) ON HOST PLANTS

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ABSTRACT

The liquid excretion and survival of the sharpshooters *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret), vectors of *Xylella fastidiosa* in citrus, were measured on various host plants as an indirect approach to assess their feeding and performance on these hosts and determine suitable plants for laboratory rearing. Adult females of *D. costalimai* showed the highest excretion rate on *Vernonia condensata* (Asteraceae). *O. facialis* excreted larger volumes on three species of *Vernonia* and on *Lantana camara* (Verbenaceae). On average, single *D. costalimai* females excreted a liquid volume equivalent to 292 times its body volume per day when feeding on *V. condensata*, whereas *O. facialis* females excreted 430 times their body volume on the same host. In contrast, the excretion rates of *D. costalimai* and *O. facialis* females on *Citrus sinensis* did not exceed 248 and 140 times their body volume per day, respectively. The mortality of adults after 96 h was lower on hosts upon which higher liquid volumes were excreted; therefore, there is a positive relationship between the excretion rate by the sharpshooters and their nutritional adequacy to hosts. *V. condensata* is a suitable host to maintain adult populations of both sharpshooters in the laboratory.

Key Words: leafhopper vectors, host plant suitability, honeydew excretion, citrus variegated chlorosis

RESUMO

A taxa de excreção de líquidos e a sobrevivência das cigarrinhas *Dilobopterus costalimai* Young e *Oncometopia facialis* (Signoret) (Hemiptera: Cicadellidae), vetoras de *Xylella fastidiosa* em citros, foram quantificadas em diferentes espécies vegetais, como forma indireta de se avaliar a adequação dessas plantas como hospedeiras das cigarrinhas para estudos ecológicos e de criação em laboratório. O maior volume de excreção líquida por fêmeas de *D. costalimai* foi observado em *Vernonia condensata* (Asteraceae). *O. facialis* excretou maiores volumes em três espécies de *Vernonia* e em *Lantana camara* (Verbenaceae). Alimentando-se em *V. condensata*, uma única fêmea de *D. costalimai* excretou, em média, o equivalente a 292 vezes seu volume corpóreo por dia, enquanto que as fêmeas de *O. facialis* excretaram 430 vezes seu volume corpóreo no mesmo hospedeiro. Em *Citrus sinensis*, as taxas de excreção de *D. costalimai* e *O. facialis* não excederam em 248 e 140 vezes o volume corpóreo por dia, respectivamente. A mortalidade dos adultos após 96 h foi menor naqueles hospedeiros onde houve maior excreção, havendo, portanto, uma relação direta entre a taxa de excreção pelas cigarrinhas e sua adequação nutricional aos hospedeiros. *V. condensata* é um hospedeiro adequado para manter populações de adultos de ambas as cigarrinhas em laboratório.

Translation provided by author

The leafhoppers *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret) (Hemiptera: Cicadellidae: Cicadellinae) are vectors of the bacterium *Xylella fastidiosa* (Roberto et al. 1996), the causal agent of citrus variegated chlorosis (CVC), a disease reported in Brazil in the late 1980s (Rossetti & De Negri 1990), which currently affects 38% of citrus trees in the state of São Paulo, Brazil (68 million plants) (Anonymous 2002).

Cicadellinae leafhoppers, commonly named sharpshooters, are usually found on plant branches, feeding in the xylem vessels of young shoots. They have well developed suction chambers that allow fluid intake even under strong negative pressure of the xylem (Purcell 1989). They extract most of the nutrients present in the ingested sap, mainly amino acids and organic acids (Andersen et al. 1989), and excrete the liquid excess through the anus. To make up for the low concentration of amino acids in the xylem sap of the plants, these insects usually ingest a large amount of liquids (Raven 1983; Purcell 1989).

Studies on insects feeding directly from the xylem fluid, e.g., the sharpshooters, rarely provide

direct results of the assimilated nutrients due to the fact that this liquid has a low chemical diversity in comparison with other plant tissues and is little likely to contain compounds of secondary metabolism (Raven 1983). Previous studies with the glassy-winged sharpshooter, *Homalodisca coagulata* Say revealed that its adaptation to the host includes high rates of assimilation of organic compounds (above 98%) and excretion of ammonia as a primary product (Andersen et al. 1989; Andersen et al. 1992). The requirement of plant nutrients varies according to the development stage of *H. coagulata*, which rarely completes its development on a single host (Andersen et al. 1989; Brodbeck et al. 1993; Brodbeck et al. 1995). According to Paiva et al. (1996) and Gravena et al.(1998), there is a clear difference between leafhopper species occurring on citrus trees and those on invasive vegetation of orchards; nevertheless, some sharpshooters that occur predominantly on the weeds are eventually trapped in the citrus canopy. Likewise, citrus sharpshooters have been found on a wide range of trees and shrubs in woody habitats adjacent to citrus orchards (J. R. S. Lopes et al., unpublished data).

The goal of this work was to develop a method to collect and measure the liquid excretion of sharpshooters, in order to evaluate feeding and survival rates of *O. facialis* and *D. costalimai* on various host plants, as an indirect approach to determine host suitability and understand the nutritional ecology of these important vectors.

Materials and Methods

The experiment was performed in a greenhouse at the Dept. of Entomology, Plant Pathology and Agricultural Zoology, University of São Paulo, Brazil. The liquid excretion of *D. costalimai* and *O. facialis* was measured on three plant species of the family Asteraceae (*Vernonia* sp., *V. condensata*, *V. polyanthes*), two of Verbenaceae (*Lantana camara* and *Aloysia virgata*), and one of Rutaceae (*Citrus sinensis*; sweet orange), which are field hosts of these sharpshooters (J.R.S. Lopes et al., unpublished data). Six-month old potted citrus trees were used. The other host plants were 3-4 months old.

Sharpshooters used in the experiment were reared on plants of *V. condensata* in a greenhouse. For collecting the liquid excretion, 1-week old adults of *D. costalimai* and *O. facialis* males and females were individually placed inside 100-ml plastic cages with lids containing ventilation holes covered by a fine fabric (Fig. 1A). The cages were attached with adhesive tape to the young branches of the plants. Feeding was allowed for 96 h; the liquid excretion accumulated in the bottom of the cages was collected daily by a 1-ml syringe, and the volume was measured (Fig. 1B). The data were transformed into liquid excretion volume produced in relation to the body volume of the insects. The body volume was determined by plunging sharpshooter adults into a known volume of liquid excretion and measuring the volume of liquid displaced. Feeding trials were run until 12 replicates were completed. Only replicates in which the insect was alive throughout the 96-h feeding period were considered for the analyses of excretion rates.

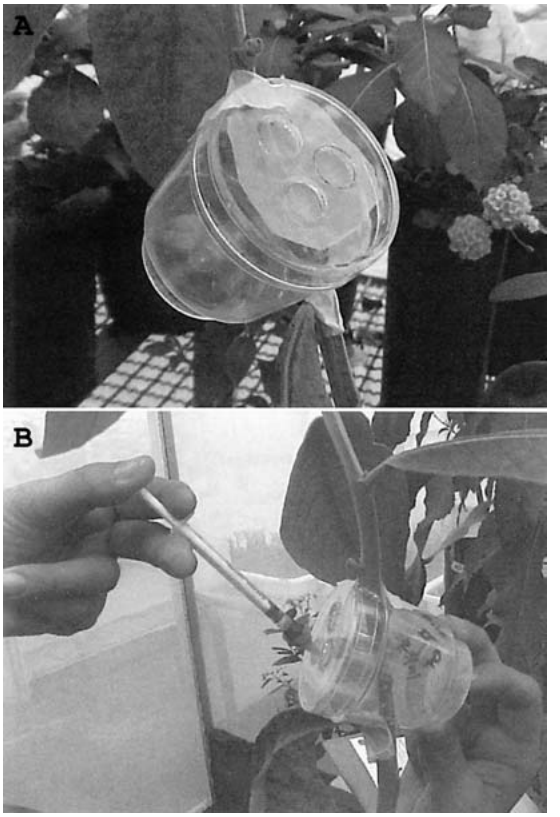

Fig. 1. A) Transparent plastic cage (100 ml) used for confinement of sharpshooters on plant stems. Lid with ventilation holes covered by a fine fabric. B) Collection and measurement of liquid volume excreted in the bottom of the cage by a 1-ml syringe.

The experimental design was in blocks completely randomized with six treatments and 12 replications. The data were analyzed using analysis of variance (ANOVA) followed by the Tukey test ($P < 0.05$).

Results and Discussion

The method developed was efficient to estimate the excretion rates of the sharpshooters. *D. costalimai* adults excreted a higher liquid volume when fed on *V. condensata* (male and female) *Vernonia* sp.(male) and *V. polyanthes*, which are plants of the family Asteraceae. On *V. condensata*,

a single *D. costalimai* female excreted up to 620 times its own body volume in a 24-h period. *D. costalimai* males and females nearly did not feed on *L. camara*, and the liquid excretion of males was null (Table 1). The same trend of higher liquid excretion on Asteraceae was verified for *O. facialis*, except for *L. camara* (Verbenaceae), upon which the excretion was equivalent to that observed on *Vernonia* sp. and *V. condensata*. In 24 h, *O. facialis* females excreted up to 900 and 990 times their body volume when fed on *V. condensata* and *L. camara*, respectively (Table 1). It should be pointed out that under field conditions *L. camara* is frequently visited by adults of *O. facialis* (Gravena et al. 1998).

The mortality of *D. costalimai* adults after 96 h was higher on *A. virgata*, *L. camara* and *C. sinensis*, and null for *V. condensata* and *Vernonia* sp. For *O. facialis* the mortality was also null when fed on *V. condensata*, *Vernonia* sp. and *V. polyanthes* (Table 2). Therefore, the host plants of the family Asteraceae appear to be nutritionally more adequate for both sharpshooters because a lower mortality and a higher liquid excretion occurred on those plants, even though the xylem sap nutrients (amino acids and sugars) considered important to the adults were not measured in this research. Milanez et al. (2001) showed that *V. condensata* is more adequate than *Citrus limonia* for the nymphal development of *D. costalimai* and *O. facialis*, because it shortens the nymphal period and increases the viability of these sharpshooters. In the present study, male and female adults of *D. costalimai* and *O. facialis* excreted much less on *C. sinensis* than on *V. condensata*, which seems to be an optimum feeding host (Table 1).

The feeding preferences of the sharpshooters might influence their competency as vectors of *X. fastidiosa*. The low rates of sap ingestion and survival on citrus by sharpshooters may theoretically reduce the chances of acquisition of *X. fastidiosa* from or inoculation to the xylem of citrus plants. This might explain in part the low transmission efficiency of *X. fastidiosa* by sharpshooters reported in citrus (Lopes 1999; Yamamoto et al. 2002); other possible factors are related to pathogen-plant or pathogen-vector interactions.

Overall, this study shows a relationship between the liquid volume excreted by *D. costalimai* and *O. facialis* adults and the nutritional adequacy of host plants. It was observed that some plants promote higher feeding and survival rates than the others. Among these hosts, *V. condensata* appears to be the most suitable to maintain adult populations of both sharpshooters in the laboratory. Further studies on oviposition and development of these sharpshooters on various host plants are necessary to understand their nutritional ecology and improve the rearing system. Previous studies showed that *H. coagulata*, a sharpshooter with similar habits, requires different hosts to complete its development (Andersen et al. 1989; Brodbeck et al. 1993; Brodbeck et al. 1995).

TABLE 1. DAILY AVERAGE AND VARIATION INTERVAL (IN PARENTHESIS) OF THE RATIO OF LIQUID VOLUME EXCRETED PER BODY VOLUME OF *DILOBOPTERUS COSTALIMAI* AND *ONCOMETOPIA FACIALIS*, WHEN FED ON DIFFERENT HOST PLANTS.

	D. costalimai		*O. facialis*	
Host plant	Male	Female	Male	Female
Vernonia condensata	196 ± 42 a[1] (80 – 520)	292 ± 60 a (160 – 620)	128 ± 51 ab (10 – 630)	430 ± 99 a (40 – 900)
V. polyanthes	134 ± 20 a (42 – 246)	84 ± 38 bc (4 – 380)	164 ± 70 ab (4 – 340)	70 ± 10 b (10 – 530)
Vernonia sp.	98 ± 40 bc (32 – 366)	48 ± 20 bc (40 – 184)	337 ± 58 a (50 – 630)	255 ± 81 a (170 – 680)
Aloysia virgata	24 ± 3 d (20 – 180)	56 ± 20 bc (20 – 520)	31 ± 12 c (2 – 140)	44 ± 24 b (1 – 260)
Lantana camara	0 d	8 ± 2 c (0 – 12)	291 ± 84 a (50 – 810)	411 ± 67 a (80 – 990)
Citrus sinensis	46 ± 2 cd (18 – 128)	120 ± 30 b (20 – 248)	36 ± 20 c (2 – 230)	32 ± 18 b (16 – 140)
ANOVA[2]				
F	4.94	9.87	8.10	11.11
df	5, 66	5, 66	5, 66	5, 66
P	<0.05	<0.05	<0.05	<0.05

[1]Means (±SEM) within columns followed by the same letter do not differ by Tukey test (P > 0.05).
[2]Analysis of variance (ANOVA) statistics; df = degrees of freedom (treatment, residue).

TABLE 2. MORTALITY (%) OF *DILOBOPTERUS COSTALIMAI* AND *ONCOMETOPIA FACIALIS* ADULTS WHEN CONFINED ON DIFFERENT HOST PLANTS FOR 96 H.

	D. costalimai		*O. facialis*	
Host plant	Male	Female	Male	Female
Vernonia condensata	0	0	0	0
V. polyanthes	25	40	0	0
Vernonia sp.	0	0	0	0
Aloysia virgata	77	50	57	40
Lantana camara	55	50	25	25
Citrus sinensis	50	57	62	40

The information obtained in this work should be useful for development and application of new vector control strategies involving trap plants or vegetation management in citrus groves.

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RELATIVE EFFECTS OF CLIMATE AND CROWDING ON WING POLYMORPHISM IN THE SOUTHERN GROUND CRICKET, *ALLONEMOBIUS SOCIUS* (ORTHOPTERA: GRYLLIDAE)

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ABSTRACT

Many factors determine the formation of flight wings in wing-polymorphic insects. Earlier studies on a cricket (*Gryllus firmus*) population producing spring and summer generations showed a declining frequency of macropterous, or long-winged, adults towards the end of a growing season. Numerous confounding factors can explain this seasonal decline, one of which is increasing mortality rates of juveniles that may otherwise emerge as macropterous adults. To test this hypothesis, we measured rates of juvenile mortality and adult macroptery in *Allonemobius socius* Scudder (Orthoptera: Gryllidae), an organism with a seasonal phenology similar to that of *G. firmus*. After rearing *A. socius* juveniles exclusively under "spring" versus "summer" conditions and at different population densities, we found that crickets reared in groups under "summer" conditions tended to emerge as macropters, with females being more likely than males to emerge long-winged. Juvenile mortality did not adequately explain the emergence pattern of macropters. Surprisingly, variation among families accounted for <1% of total variation in frequency of long-winged adults. Thus, seasonal climate, followed by population density, and then their interaction with each other appear to be the three major determinants of wing morph frequencies in *A. socius*. We discuss the possible adaptive significance of wing polymorphism in insects with respect to habitat persistence and mating success.

Key Words: migration, habitat persistence, polyphenism, crowding, wing dimorphism, plasticity

RESUMEN

Muchos factores determinan la formación de las alas de vuelo en insectos de alas polimórficas. Estudios anteriores sobre una población del grillo (*Gryllus firmus*) produciendo generaciones en la primavera y en el verano mostraron una frecuencia diminuyendo de adultos macrópteros, o de alas largas, acercandose al final de la estación de crecimiento. Numerosos factores componentes pueden explicar esta declinación estacional, uno de ellos es el aumento en la tasa de mortalidad de los juveniles que de otra manera emergirán como macrópteros adultos. Para probar esta hipótesis, nosotros medimos las tasas de mortalidad juvenil y la macroteria (el estado de alas largas) de los adultos en *Allonemobius socius* Scudder (Orthoptera: Gryllidae), un organismo con una fenologia estacional similar a la de *G. firmus*. Después de criar los juveniles de *A. socius* exclusivamente bajo condiciones de "primavera" versus "verano" y en diferentes densidades de poblacion, nosotros encontramos que los grillos criados en grupos bajo condiciones de "verano" tendian a emerger como macrópteros, y fué más probable que las hembras emergen con alas largas que los machos. La mortalidad juvenil no explicó adecuadamente el patrón de emergencia de los macrópteros. Sorprendentemente, la variación entre las familias contaba por <1% de la variación total en la frecuencia de adultos con alas largas. Asi, el clima estacional, seguido por la densidad de la población, y después la interacción entre ellos parecen ser los tres mayores determinantes en la frecuencia de las diferentes formas de alas en *A. socius*. Nosotros discutimos el posible significado adaptivo derivado del polimorfismo de alas en insectos al respecto de la persistencia de habitat y el éxito en el apareamiento.

The independent evolution of wings among several animal taxa (Kingsolver & Koehl 1994) is due, in part, to the apparent benefits of flight. Flight-capable organisms can easily colonize peripheral areas of their current habitat, as well as migrate to more distant and, perhaps, novel environments in search of food and mates. By enhancing mobility and dispersal, flight undoubtedly

contributed to the remarkable diversification of insects (Roff & Fairbairn 1991; Rankin & Burchsted 1992; Kingsolver & Koehl 1994).

Developing and maintaining the flight apparatus, however, often carries a cost. For example, long-winged (or macropterous) females of the sand cricket, *Gryllus firmus*, reach reproductive age at a later date and have lower lifetime fecundities when compared to their short-winged counterparts (Roff 1990a). In the brown planthopper, *Nilaparvata lugens*, macroptery is associated with longer egg-to-adult development time and lowered male mating success (Novotny 1995). Such trade-offs between flight wings and other traits closely associated with fitness allows one to view flight ability as itself a fitness-determining trait along the same lines as growth rate and fecundity. Thus, not only is wing polymorphism interesting for its own sake, but also for its apparent ties to life history and life cycle evolution (Roff & Fairbairn 1991; Rankin & Burchsted 1992; Kingsolver & Koehl 1994; reviewed extensively in Dingle 1996).

Apart from being genetically determined (Masaki & Walker 1987; Mousseau & Roff 1989; Roff 1990a, 1990b), flight wings can develop in response to numerous environmental factors. For example, warm temperatures and long-day conditions typical of summer tend to produce macropterous adults, e.g., in crickets and grasshoppers (Tanaka 1978; see also Masaki & Walker [1987], and references therein) and in *Gerris* species (Vepsäläinen 1978, in Dingle 1996). Conditions of crowding and food shortage also contribute to variation in the frequency of wing morphs in insect populations (Tauber et al. 1986; Walker 1987).

In their exhaustive survey of one population of the sand cricket, *Gryllus firmus*, Veazey et al. (1978) found that the frequency of macropterous adults caught in pitfall traps was lower in the summer than in the spring brood. However, such a pattern can be attributed to a number of confounding and interacting variables, including: (1) migration of flight-capable, macropterous adults away from the sampling site; (2) differential mortality of presumptive macropters and micropters, due perhaps to intraspecific competition for food or space; (3) increased predation by insectivores maturing later in the growing season; (4) shedding of flight wings by individuals that emerged earlier in the season as macropterous adults (though this phenomenon has not been reported for *G. firmus*); (5) a genetically fixed seasonal phenology for macropters and micropters, e.g. macropterous adults from only the spring brood always producing offspring that always emerge a year later (in the following spring) as long-winged adults; (6) the emergence of 2nd-generation, or summer brood, juveniles as short-winged rather than macropterous adults; and (7) a deficiency in the sampling methods used by Veazey et al. (1978). Investigations since then have elaborated some of the genetic and physiological mechanisms that contribute to variation in *G. firmus* wing morph frequencies (Roff 1990a, 1990b; Zera et al. 1998). But surprisingly, very little empirical work has been done to tease out which ecological factors are most responsible for this pattern of naturally occurring wing polymorphism (cf. Roff 1994a; Crnokrak & Roff 1998).

The current study addresses the hypothesis that differential juvenile mortality explains variation in wing morph frequency in wing polymorphic insects (see Factor 2 above). Both spring and summer broods of the southern ground cricket, *Allonemobius socius* Scudder (Orthoptera: Gryllidae), occur at high densities throughout the southeastern region of North America, and experience a variety of seasonal temperatures and day-lengths associated with their widespread latitudinal and altitudinal distribution (Howard & Furth 1986; Mousseau & Roff 1989). Moreover, like *G. firmus*, both field and laboratory populations of *A. socius* produce a mixture of short- and long-winged adults, with the latter form also exhibiting variation in flight propensity (A.E.O., personal observation). Thus, *A. socius* is useful for investigating the genetic and environmental factors responsible for variation in wing morph frequencies in natural insect populations.

Materials and Methods

Cricket Stocks

All individuals used in this experiment were first-generation, laboratory-reared descendents of crickets caught as juveniles from a wet, grassy field adjacent to a greenhouse on the University of South Carolina—Columbia. Before the start of the experiment, all crickets and their eggs were incubated, reared, and maintained under conditions simulating a hot, summer day (31°C, 15 h day-length, >60% relative humidity) in Columbia, South Carolina, U.S.A. (Wood 1996). Voucher specimens have been sent to J. C. Morse of the Clemson University Arthropod Collection.

Experimental Design

Individually reared juveniles from 23 full-sibling families were housed in clear plastic petri plates (diameter = 100 mm) each provisioned ad libitum with pulverized cat chow, chopped carrots, water, and shredded unbleached paper towels for cover. Group-reared juveniles from 31 full-sibling families were housed in $9 \times 9 \times 8$ cm clear plastic cages that were similarly provisioned. Left-over cat chow and carrots were changed every 2 to 3 days.

We used a double split-brood design, in which one-half of a cohort of newly hatched juveniles from each family was randomly assigned to a

"summer" (31°C, 15 h day-length) rearing environment, while the other half was reared in a "spring" (24°C, 11 h day-length) environment. Within a seasonal environment, group-reared juveniles were then assigned to either a high population density (14 to 21 juveniles per cage) or low population density (3 to 6 juveniles per cage) treatment. Among-family variation in maternal fecundity, egg-hatching rate, and juvenile survivorship precluded assignment of exactly equal numbers of individuals to replicate cages (in each population-density treatment). Because *A. socius* will consume dead conspecifics when available, we minimized scavenging by removing dead individuals without replacement. All surviving juveniles were reared to adulthood exclusively in the environment to which they were initially assigned.

Scoring Macroptery and Juvenile Survivorship

Only macropterous adults possess the ivory-colored flight wings that extend from beneath the beige-black tegmina, or outer wings (Fig. 1). We scored adults <3 d after the final molt as macropterous if they emerged with flight wings intact. In group-reared crickets, incidence of macroptery was then calculated as the number of macropterous adults divided by the total number of adults from a given replicate cage. Similarly, incidence of macroptery in crickets reared in isolation was calculated as the number of macropterous adults divided by the total number of adults that were reared in petri plates within a given seasonal environment.

Juvenile survivorship of group-reared crickets was calculated as the number of emerging adults (regardless of wing morph) divided by the total number of nymphs from a given replicate cage. Similarly, juvenile survivorship for crickets reared in isolation was calculated as the total number of petri plates that yielded (long- or short-winged) adults divided by the original number of petri plates used to rear cricket nymphs individually.

Statistical Analyses

Because the macroptery data did not satisfy the normality assumptions for valid parametric analyses, we turned to a nonparametric, van der Waerden normal scores analysis to test for sex-specific differences in proportion of macropters for each treatment. We performed the NPAR1WAY procedure in SAS For Windows, Version 6.12 (SAS 1989). As discussed fully in Conover (1999), a van der Waerden analysis achieves asymptotic relative efficiency, or A.R.E. (~statistical power), comparable to that of para-

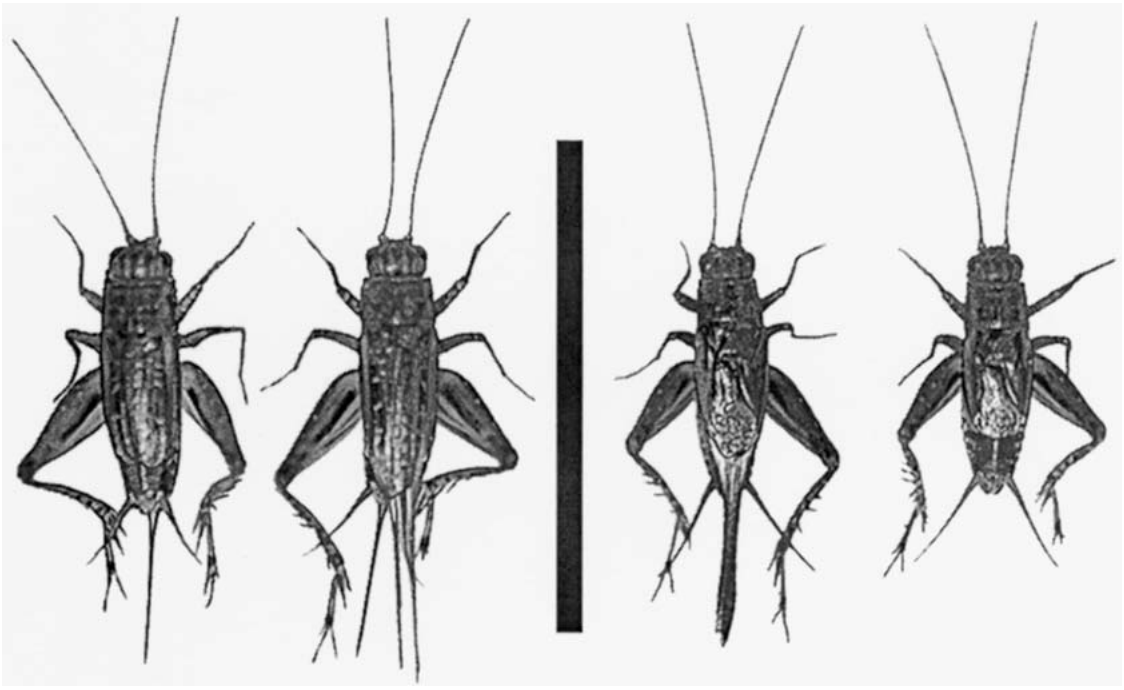

Fig. 1. Wing polymorphism in *A. socius*. Females are distinguished from males by the presence of a sword-like ovipositor protruding from the posterior end of the abdomen. Note the flight wings in long-winged adults—the two individuals flanking the vertical black bar—extend from beneath the darker tegmina (i.e., outer wings). Length of vertical black bar is 20 mm.

metric statistical tests, e.g., F-test, when data satisfy normality assumptions, and greater A.R.E. when data are non-normal. We adjusted the resulting probability values using the sequential Bonferroni method in order to maintain an experiment-wide Type I error rate of ≤5% for all pairwise comparisons (Rice 1989).

We partitioned total variance in both the frequency of macropters and juvenile survivorship via the restricted maximum likelihood (REML) method available in the VARCOMP procedure of SAS for Windows 6.12 (SAS 1989). Although the VARCOMP procedure does not provide P-values for estimated variance components, it allows a quantitative description of importance of each biotic and abiotic factor to observed macroptery patterns in *A. socius*. The choice of REML as a method for estimating variances is justified by its common use in modern quantitative genetic studies, e.g., Shaw (1987), Meyer and Hill (1991), and Ferreira et al. (1999).

Results

Compared to the spring rearing treatment, the summer rearing environment tended to produce a higher proportion of long-winged adults (Fig. 2). Differences in macroptery rates between the sexes appeared only at high population densities (Table 1), with more females than males emerging as long-winged adults (Fig. 2).

The largest contributing factor to variance in macroptery rates was juvenile rearing climate, followed by the interaction of rearing season with population density (Table 2). Family origin (nested within population density since not all of the families were represented in the population-density manipulations) contributed <1% to variance in macroptery rates (Table 2).

Juvenile survivorship was high across all rearing treatments. As expected, crickets reared in isolation had higher survival rates (~85% in both spring and summer rearing environments) than those reared in groups, with high population densities resulting in the lowest survival rates (between 58-78%). At a given population density, juvenile survival rates were similar for both summer and spring rearing conditions (Fig. 3), suggesting no effect of seasonal climate and no interaction between seasonal climate and population density on juvenile survivorship (Table 2).

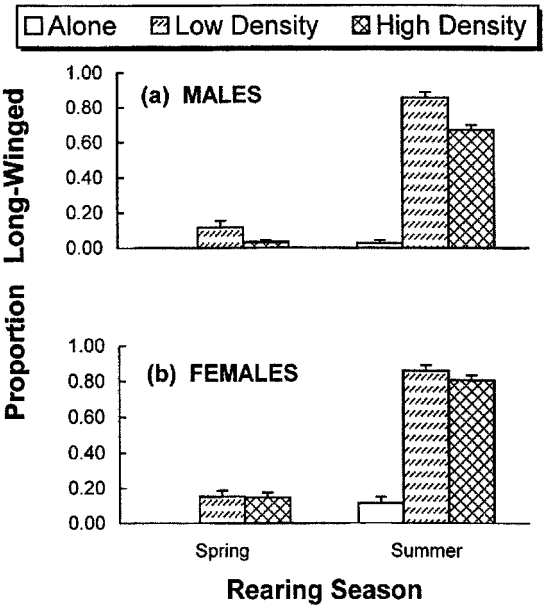

Fig. 2. The effects of rearing season and population density during juvenile development on mean (±SE) incidence of macroptery in *A. socius* males and females. All juveniles reared in isolation and under spring-like conditions emerged as short-winged adults.

We found that the largest contributors to variation in juvenile survivorship (aside from experimental error) were population density and family origin (Table 2). There were no significant interactions between either of these factors with rearing season.

Discussion

Both seasonal climate and population density during juvenile development affect frequency of macropters in *A. socius*. In our study, high temperatures and long day-lengths typical of the summer

Table 1. Effect of rearing environment on the difference in the proportion of long-winged females to long-winged males.

	Spring-reared[a]	Summer-reared
Reared alone[b]	N/A	4.739
Reared at low population density	1.569	0.001
Reared at high population density	13.692*	13.139*

[a]Numbers are test statistics, T1, from a nonparametric van der Waerden one-way normal scores analysis (Conover 1999) with one degree of freedom.
[b]All juveniles reared alone under spring-like conditions emerged as short-winged adults.
*Statistically significant at experiment-wide $\alpha = 5\%$.

TABLE 2. RESTRICTED MAXIMUM-LIKELIHOOD VARIANCE-COMPONENTS ESTIMATION OF RELATIVE CONTRIBUTION TO INCIDENCE OF LONG-WINGED, EMERGING ADULTS AND TO JUVENILE SURVIVORSHIP.

Observed variance component	Incidence of macroptery	Juvenile survivorship[a]
(1) Rearing season	40.1%	0.9%
(2) Population density	12.2%	38.8%
(3) Family (population density)[b]	0.1%	21.5%
(4) Sex (population density, family)[c]	0.8%	N/A
Interaction of 1 and 2	25.5%	0.0%
Interaction of 1 and 3	1.1%	0.0%
Interaction of 1 and 4	1.1%	N/A
Error	19.1%	38.8%
Total	100%	100%

[a]Sex of newly hatched juveniles could not be determined non-invasively because secondary sexual traits appear only in the middle-to-late stages of development. Hence, variance in juvenile survivorship due to sex and to interaction between sex and rearing season cannot be estimated.

[b]Families experienced both rearing seasons, but not all population-density treatments.

[c]Because a few families produced single-sex progeny, sex is nested within family, which in turn is nested within population density.

season led to greater numbers of macropterous males and females (Fig. 2; see comparable results in Tanaka 1978). Population density greatly compounded the effect of the summer rearing environment in producing macropterous adults, especially with crickets reared in groups (Fig. 2). At low population densities, the proportion of macropterous adults increased as climate changed from spring- to summer-like (Fig. 2; but see Walker 1987). At high population densities, we found a reduced proportion of macropterous adults (Fig. 2), a result that may be due to intraspecific competition for space and nutrients (discussed in Walker [1987] and Zera & Tiebel [1988]).

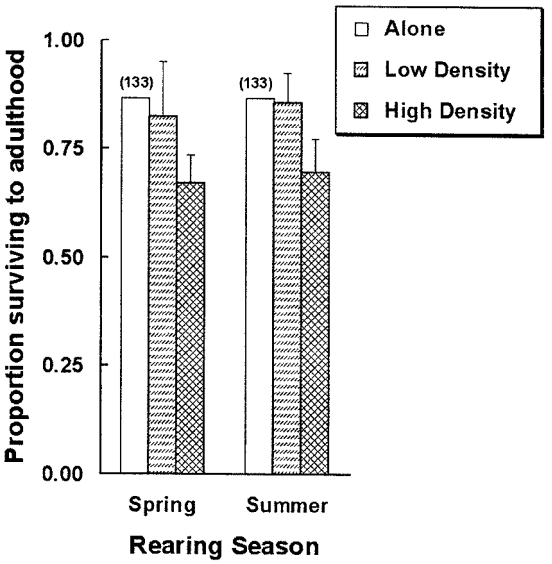

Fig. 3. Juvenile survivorship of *A. socius* at different rearing densities and seasonal climates. Column height represents the mean (±SE) proportion of newly hatched juveniles that eventually emerged as adults. Numbers in parentheses above each clear column indicates total number of individually reared *A. socius* juveniles scored for that treatment.

Differences in juvenile survivorship among our rearing treatments do not adequately explain the variation in incidence of macroptery. Though juvenile survivorship appeared inversely related to population density in the rearing cages, the differences in juvenile survivorship among the population-density treatments were similar between spring- and summer-rearing treatments (Fig. 3), suggesting that seasonal climate had little effect on *A. socius* juvenile survivorship. More importantly, the pattern of juvenile survivorship did not parallel that of adult macroptery rates among treatment groups (Fig. 2). Thus, wing length variation in *A. socius* appears to be a response to seasonal climate and population rearing density (Fig. 2), and does not appear to reflect differences in juvenile survivorship.

The extent to which population density affects the timing of flight wing removal in *A. socius* is not known. It is possible that macropterous *A. socius* adults shed their flight wings <3 d post-eclosion, which would have introduced a downward bias in our method of scoring macroptery. In other words, the incidence of long-winged morphs in our high cage-density treatment may actually be higher than was observed in this study (Fig. 2). Notwithstanding this potential bias, the observed pattern of juvenile survivorship does not parallel that of macroptery rates.

Probabilistic models, e.g. in Roff (1975), "adaptive coin-flipping" of Kaplan and Cooper (1984), and "stochastic polyphenism" of Walker (1986), have been proposed to explain wing polymorphism in crickets and other flight-capable insects.

One simple prediction under probabilistic theory is that the proportion of long-winged adults will reflect the probability of those individuals experiencing a future environment that selects for flight. Unfortunately, the present results cannot confirm such predictions, nor can they validate probabilistic models in general because environmental variation in the wild (as experienced by *A. socius* juveniles of the parental generation in this study) would have been impossible to mimic in a laboratory setting. Critical tests of probabilistic models about macroptery may inevitably involve the use of more closely monitored rearing environments and isogenic lines.

The greater propensity of females than males to possess flight wings (Fig. 2) may be attributed, in part, to sex-specific reproductive behavior. In many crickets, sexually receptive females often travel some distance to locate the stationary, calling male (Loher & Dambach 1989). Presumably, the fitness gain from flight-aided mate-locating behavior more than compensates for the cost of developing the necessary flight apparatus in *A. socius* females.

Macroptery in male crickets, on the other hand, can impose a tremendous fitness cost in that macropterous males tend to be less successful than their short-winged counterparts in attracting females (Crnokrak & Roff 1995), although this has not been tested for *A. socius*. As well, male crickets probably have greater mating success when they remain in their natal habitat rather than fly to unknown destinations where mating opportunities may be scarce or absent (Walker 1986).

The greater incidence of macroptery in *A. socius* females than males might also reflect the role of habitat persistence in maintaining wing dimorphism (Denno et al. 1991; Roff 1994b). To the best of our knowledge, the field from which we collected crickets was watered daily and mowed every 3-6 wk during late-spring and throughout the summer months by campus grounds crew. The environmental disturbance caused by mowing could have augmented selection for late-summer dispersal, especially in female *A. socius*, since females can store sperm from previous matings. In such case, a female cricket has little reproductive "need" for males once she has reached the less affected periphery of her natal habitat. Thus, in patchy and temporary environments, natural selection might have acted to maintain the flight apparatus in a flight-capable female just long enough for her to escape a disturbed or deteriorating patch of habitat, and to colonize areas more conducive to oviposition and optimal development of her offspring during the regular growing season (Southwood 1962; Dingle 1996; Denno et al. 1991; Roff 1994b).

The apparent synergy between summer climate and moderate-to-high rearing density in producing long-winged *A. socius* suggests that, in bivoltine populations, long-wingedess is more common in the 2nd generation than in the first. In the bivoltine life cycle of *A. socius*, the 1st generation is comprised of individuals that had survived the past winter as diapausing eggs and then hatched out in spring. These 1st generation juveniles develop to adulthood through spring and early summer, by which time they mate and produce non-diapausing eggs that hatch out immediately (Walker & Masaki 1989; Mousseau & Dingle 1991; Olvido et al. 1998). Thus, 2nd generation juveniles appear likely to experience the macroptery-inducing summer season, as well as higher population density resulting from the presence of 1st generation adults and other newly hatched 2nd generation juveniles. Experiments are under way to assess this prediction.

On the other hand, Veazey et al. (1976) showed that macroptery rates declined from summer through autumn in a Florida population of *G. firmus*. Proximate mechanisms that can explain such a pattern, e.g. predation pressure and migration of macropters from field sites, have yet to be fully explored in this and other insects.

In this current study, we found no evidence that would suggest differential juvenile survival affects macroptery rates in *A. socius*. That is, differences in wing morph frequencies between spring- and summer-reared full-siblings of *A. socius* are not likely due to differences in juvenile mortality, but instead may result from a response to seasonal climate and population density during juvenile growth. However, the generality of emergence patterns in *A. socius*, like in *G. firmus*, will require further and more detailed investigation of seasonal phenology in other wing polymorphic organisms.

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OVIPOSITION AND LARVAL SURVIVAL OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) ON SELECT HOST PLANTS

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ABSTRACT

In a preliminary survey in four commercial ornamental nurseries in south Florida (1998), *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) egg masses, feeding damage, or adults occurred on numerous field-grown ornamental plant species. Live oak (*Quercus virginiana* Mill.), silver buttonwood (*Conocarpus erectus* L. variety *sericeus* Fors. Ex DC), and black olive (*Bucida buseras* L.) had the highest percentage of plants with egg masses. Adult feeding damage was found on all examined plants of dahoon holly (*Ilex cassine* L.), cocoplum (*Chrysobalanus icaco* L.), black olive, live oak, *Bauhinia* sp., and *Cassia* sp. Oviposition of *D. abbreviatus* was evaluated in no-choice, two-choice, three-choice and multiple–choice caged tests. In no-choice tests, silver buttonwood had the highest mean number of egg masses. In two-choice tests, egg masses were laid on all plant species tested but there were significantly more egg masses on silver buttonwood than the alternate choice. The number of egg masses in the three-choice tests was low and there were no significant differences among the plant species tested. As in the no-choice and two-choice tests, significantly more egg masses were found on silver buttonwood in multiple-choice tests. Survival of larvae and their effect on plant growth was examined on several commonly grown plant species in southern Florida. Larval survival was highest on silver buttonwood and *Sorghum sudanense* Pers (sorghum-sudan) compared with other plant species. Root and/or total biomass was significantly reduced on green bean (*Phaseolus vulgaris*), silver buttonwood, Tahiti lime (*Citrus aurantifolia*), and sorghum-sudan.

Key Words: root weevil, oviposition, host preference, larval survival

RESUMEN

En un muestreo preliminar realizado en 1998 en cuatro viveros de plantas ornamentals localizados en el sur de Florida, se demostró que *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) estaba asociado oviposición, daño al follaje, o en base a la presencia de adultos en varias especies de plantas ornamentals. Las especies de plantas que presentaron el mayor porcentaje de oviposición fueron el roble, (*Quercus virginiana* Mill), el arbol plateado del botón (*Conocarpus erectus* L.,) variedad *sericeus* y la bucida *(Bucida buseras* L). Se observó consumo del follaje por adultos en todas las plantas muestreadas de las siguientes especies, acebo (*Ilex cassine* L.), icaco (*Chrysobalanus icaco* L.), bucida, roble, casco de vaca *Bauhinia* sp., y *Cassia* sp. Se evaluó la oviposicion de *Diaprepes* mediante experimentos en jaulas donde se ofrecieron una opción de planta, dos opciones de plantas, tres opciones de plantas, y varias opciones de plantas. En los experimentos con una sola opción de plantas, el arbol plateado del boton obtuvo el mayor número de huevos. En experimentos con dos opciones de plantas, se encontraron posturas en todas las plantas, pero mas en el arbol plateado del boton comparado con otras plantas. Cuando se ofrecieron 3 opciones de plantas el número de posturas por planta fue bajo, y no hubo diferencias entre las especies expuestas. En las pruebas de opciones multiples de plantas hospederas, el arbol plateado del botón tuvo mayor oviposición que las otras especies. La supervivencia de las larvas y su efecto en el crecimiento de examinado en varias plantas cultivadas en el Sur de la Florida. La supervivencia de las larvas fue mayor en raices del arbol plateado del botón, y sorgo sudanés comparado con la supervivencia en las raices de otras especies de plantas. *Diaprepes abbreviatus* redujo significatinamente el peso de las raíces y el peso total de frijol verde (*Phaseolus vulgaris*), arbol plateado del botón, lima acida (*Citrus aurantifolia*) y sorgo sudanés.

Translation provided by author.

The root weevil, *Diaprepes abbreviatus* (L.), native to the Caribbean Islands, is believed to have entered Florida from Puerto Rico on a shipment of ornamental plants (Woodruff 1985). It was first reported in an Apopka nursery in 1964 (Woodruff 1968) and has spread throughout many counties

in Florida. *Diaprepes abbreviatus* is associated with at least 270 plant species including several important and economic crops grown in Florida such as citrus, ornamentals, and sugar cane (Simpson et al. 1996). In citrus groves the cost of control and losses incurred by *Diaprepes* root weevils exceed $1,200 per acre. This pest infests approximately 60,000 acres of citrus at an annual cost of about $72 million to the Florida citrus industry (Stanley 1996). *Diaprepes* is currently attacking many ornamental nursery plants, which has resulted in restrictions on the movement of plant material from areas infested with the weevil. Quarantine treatments can be expensive, labor intensive and time-consuming. Sometimes there are losses in sales and customers because there are no known treatments acceptable for quarantine and plants cannot be shipped to a particular location. Twenty-four Florida counties were known to be infested as of April 2001 (Michael C. Thomas, Florida Department of Agriculture and Consumer Services, pers. comm.).

Adult weevils feed on plant foliage, often leaving a characteristic pattern of notches around leaf edges. Female weevils lay clusters of eggs between leaves and protect them by secreting a sticky substance that cements the leaf surfaces together (Fennah 1942; Woodruff 1968; Adair et al. 1998). The number of eggs per egg mass varies but on average is approximately 50 eggs. One female may lay as many as 5,000 to 29,000 eggs during her three to four month lifespan (Wolcott 1936; Beavers 1982). Neonates hatch from the eggs in 7 to 10 days, fall to the soil surface, and burrow into the soil seeking out plant roots on which to feed. The larvae remain in the soil 8 to 12 months where they complete development to the adult stage. Adult weevils live 4 to 5 months, but often half of this time is spent below the surface of the ground (Wolcott 1936). In Florida, there are overlapping generations with two peak adult emergence periods in the spring (May-June) and fall (August-September) (Beavers & Selhime 1976).

Adult weevils can cause moderate to severe defoliation of host plants; larval feeding can kill hosts. In some plants, larvae girdle the taproot, which reduces nutrient uptake, and ultimately kill the plant (Quintela et al. 1998). Additionally, larval root-feeding injury also provides an avenue for microbial infections such as *Phytophthora* and *Fusarium* (Knapp et al. 2000; Nigg et al. 2001a). Several larvae can cause serious decline of established citrus trees and it has been speculated by researchers that one larva is capable of killing a young citrus tree.

The objectives of this research were to evaluate host preferences for oviposition and to determine the presence of *D. abbreviatus* eggs, adults, or adult feeding damage in a preliminary survey of field-grown ornamentals, and to evaluate larval survival and root consumption of various plant species.

Materials and Methods

Field Survey

A preliminary survey for the presence of adult *D. abbreviatus*, feeding damage and egg masses was conducted over a 2-day period in four commercial, field-grown ornamental nurseries in Miami-Dade County, Florida. Three plant rows were randomly selected from each field and two people inspected all plants within the three rows for 5 minutes per plant. The presence or absence of adults, feeding damage, and egg masses on each plant were recorded. All sites contained a diversity of ornamental plants with moderate to high populations of *D. abbreviatus*.

No-Choice Oviposition Tests

Three tests were conducted to compare oviposition on four plant species when there was no choice in host plant. In each test, eight plants of the same species were placed in a screen cage (1.8 × 3.7 × 1.8 m). Each plant was planted in a 7.6 liter container with Pro-Mix 'BX' (peat-based growing medium) potting media and exhibited new leaves at the time of the experiment. In Test 1, the plants evaluated were *Conocarpus erectus* L. variety *sericeus* Fors. Ex DC (silver buttonwood), *Manihot esculenta* Krantz (cassava), *Carica papaya* L. (papaya), and *Xanthosoma* sp. (malanga). In Test 2, the plants evaluated were silver buttonwood, *Sorghum sudanense* Pers (sorghum-sudan), *Persea americana* Mill. (avocado), and *Chrysophillum oliviforme* L. (satinleaf tree). In Test 3 the plants evaluated were silver buttonwood, *Solanum tuberosum* (white potato), *Pennisetum purpureum* Schumach (elephant grass), and *Zea mays* L. (sweet corn). Fifty male and fifty female, adult *D. abbreviatus* were released in each cage. The number of adults used was selected to ensure some oviposition. Adult weevils were field collected from mixed plant species. The adult weevils were maintained in cages with *Conocarpus erectus* L. (green buttonwood) before use for approximately 24 hours. Each treatment was replicated four times. The number of egg masses per plant was recorded 7 days after the adults had been released into the cages, which was sufficient time for the weevils to lay eggs. The number of eggs per egg mass were not counted.

Two-Choice Oviposition Tests

Two tests were conducted in which adults were given a choice of silver buttonwood or another plant host (each planted in a 7.6 liter containers with ProMix potting media) on which to oviposit. In the first test the alternate plant was *Citrus aurantifolia* (Christm.) Tahiti lime, and in the second test the alternate plant was sorghum-sudan.

In each test, eight plants (four of each species) were placed in a screen cage (2 × 4 × 2 m). Each test was replicated four times. Plant species within a cage were placed in an alternating pattern. One hundred field-collected adult *D. abbreviatus* (50 female; 50 male) were released into each cage. Each plant was examined for number of egg masses as above.

Three-Choice Oviposition Tests

Three plant species were evaluated in a choice test for oviposition preference by *D. abbreviatus*. The plant species included *Citrus sinensis* L. sour orange, Tahiti lime, and silver buttonwood each grown in a 7.6 liter containers in a Pro-Mix 'BX' (peat-based growing medium) potting soil. The containers were placed on five raised beds in a screen house (12.2 × 18.3 m). Beds were 16.5 × 0.7 m with 1.3 m between beds. Twenty-four silver buttonwood were evenly spaced on each of the two outside beds with 0.61 m between plants. On the three inner beds, ten orange and ten lime trees were alternated with two buttonwood plants at the end of each bed totaling 24 plants per bed. Five hundred field-collected adult weevils from plant species not included in the test were released inside the screen house in the late afternoon on day 1. The male-female ratio was 1:1. The adult weevils were released along the centerline of the screen house perpendicular to the beds. The number of egg masses per plant was recorded on day 8.

Multiple-Choice Oviposition Test

Plants of seven species were each planted in a 7.6 liter container with Pro-Mix 'BX' (peat-based growing medium) potting soil. The plants included silver buttonwood, lime, elephant grass, sorgham-sudan, sweet corn, malanga, and white potato. Five replications of each plant were placed in a randomized complete block design in a screenhouse (12.2 m × 18.3 m). Five hundred adult *D. abbreviatus* were collected from the field from hosts other than those in the test and released inside the screen house. Ten days after the release, plant leaves were checked for egg masses.

Larval Survival and Root Consumption

Survival of larvae on different host plants and their effect on the plant was measured. Treatment containers were infested with 50 neonate *D. abbreviatus*. Neonates were collected from eggs produced by field-collected adults held in cages. For each plant species, a paired comparison of infested and not infested plants was conducted. Eight plant species were tested; *Phaseolus vulgaris* L. (green bean), silver buttonwood, lime, malanga, satinleaf, sorghum-sudan, cassava and *Ilex cassine* L. (dahoon holly). Each plant was planted in a 7.6 liter container with Pro-Mix 'BX' (peat-based growing medium) and maintained in a greenhouse. Replications for each plant species varied between 5 and 10. Green bean and sweet corn were evaluated 2 months after infestation. Silver buttonwood, Tahiti lime, malanga, satinleaf, and sorghum-sudan were evaluated 3 months after infestation. Larval survival was also evaluated on silver buttonwood, Tahiti lime and malanga 6 months after infestation. Plant height, fresh and dry weight of roots and total biomass, and the number and weight of surviving larvae were recorded. Comparisons were made between infested plants and not infested plants.

Data in all of the oviposition choice tests except the two-choice test and the larval survival tests were subjected to analysis of variance with the means compared by the Student-Newman-Keuls Range Test (SAS 1999-2001). Data from the two-choice oviposition test and all the larval feeding tests were subjected to a *t*-test (SAS 1999-2001).

Results and Discussion

Field Survey

Numerous field-grown ornamental plant species were examined for the presence of adults, feeding damage, or egg masses (Table 1). The results of this survey are preliminary but are consistent with previous surveys or plant host lists (Simpson et al. 1996; Knapp et al. 2000) that indicate that *Diaprepes* will feed and lay eggs on a wide host range of ornamental plants. The survey conducted by Simpson et al. (1996) is a compilation of specimen identification reports submitted to the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, from 1964 through 1995 in addition to any host records listed in the scientific literature from 1898 to 1995. Knapp et al. (2000) also compiled a list of plant hosts from the scientific literature but there is no information about the type of association with the host plant (i.e., adult host, larval host, etc.). The current survey is a snapshot of adult weevil presence, host plant damage, and presence of egg masses in four commercial, field-grown ornamental nurseries in south Florida. The plant species in this survey with the highest percentage of plants with egg masses were live oak, *Quercus virginiana* (46.2%), silver buttonwood (41.2%), and black olive, *Bucida buseras* (33.3%). All of the dahoon holly, cocoplum, *Chrysobalanus icaco*, black olive, live oak, *Bauhinia* sp. and *Cassia* sp. plants evaluated had adult feeding damage. Plant species with the highest number of adults per plant include dahoon holly, black olive, and *Bauhinia* sp. All plant species in the survey with egg masses, adults or feeding damage, except *Jacaranda mimosiflia* (jacaranda), *Clusia rosea* (autograph tree), and

TABLE 1. PRELIMINARY SURVEY OF ADULTS, FEEDING DAMAGE, AND EGG MASSES OF *Diaprepes abbreviatus* ON CULTIVATED NURSERY PLANTS IN MIAMI-DADE COUNTY, FL, SEPTEMBER 3-4, 1998.

Plant family	Plant species (common name)	No. plants examined	No. plants with egg masses	No. plants with feeding damage	No. plants with adults present
Agavaceae	*Cordyline terminalis* (ti plant)	3	0	2	0
	Dracaena marginata (Dracena)	2	0	0	0
Aquifoliaceae	*Ilex cassine* (dahoon holly)	8	2	8	7
Bignonaceae	*Tabebuia heterophylla* (pink trumpet tree)	5	0	0	0
	Tabebuia caraiba (silver trumpet tree)	2	0	1	0
	Jacaranda mimosifolia (jacaranda)	5	0	1	1
Boraginaceae	*Cordia sebestena* (geiger tree)	1	0	1	0
Burseracea	*Bursera simaruba* (gumbo limbo)	8	0	5	4
Chrysobalanaceae	*Chrysobalanus icaco* (cocoplum)	10	0	10	2
Combretaceae	*Conocarpus erectus* var. *sericeus* (silver buttonwood)	17	7	10	6
	Bucida buseras (black olive)	9	3	9	7
Cycadaceae	*Cycas revolute* (king sago)	5	0	0	0
Fagaceae	*Quercus virginiana* (live oak)	13	6	13	4
Guttiferae	*Calophyllum braziliense* (Brazilian beauty leaf)	25	3	11	1
	Clusia rosea (autograph tree)	11	0	10	1
Leguminosae	*Bauhinia* sp.	7	0	7	5
	Cassia sp.	31	1	31	12
Lythraceae	*Lagerstroemia* sp. (crape myrtle)	11	0	6	0
Meliaceae	*Swietenia mahogany* (mahogany)	6	1	4	1
Myrtaceae	*Eugenia* sp.	5	0	0	0
Musaceae	*Strelitzia nicolai* (bird-of-paradise)	5	0	0	0
Oleaceae	*Ligustrum* sp. (privet)	1	0	1	0
Palmae	*Cocos nucifera* (coconut palm)	9	2	1	0
	Phoenix roebelinii (pygmy date palm)	38	4	20	1
	Acoelorraphe wrightii (Everglade palm)	1	0	0	0
	Livistona chinensis (Chinese fan palm)	1	0	0	0
	Vetchia merrillii (Christmas palm)	18	0	0	1
Sapindaceae	*Litchi chinensis* (lychee)	2	0	2	0
Sapotaceae	*Chrysophyllum olivivorme* (satinleaf)	2	0	1	0

Cocos nucifera (coconut palm), have previously been reported as being associated with *D. abbreviatus* (Simpson et al. 1996; Knapp et al. 2000).

No-Choice Oviposition Tests

In two of the three tests conducted, silver buttonwood had the highest mean number of egg masses ranging from 1.75 to 4.12 egg masses per plant (Table 2). Only two plant species (malanga and satinleaf) had no egg masses.

In Test 1, significantly more egg masses were found on cassava and silver buttonwood leaves compared with papaya and malanga (F = 6.10; df = 3, 28; P = 0.0025) (Table 2). A similar result was seen in Test 2. More egg masses were found on silver buttonwood and sorghum-sudan compared with avocado (West Indies cultivar) and satinleaf (F = 8.26; df = 3, 28; P = 0.0004) (Table 2). In Test 3, the mean number of egg masses did not significantly differ among plant species (F = 1.90; df = 3, 28; P = 0.1525). However, there were approximately twice as many egg masses on silver buttonwood as on the alternate hosts (Table 2).

Two-Choice Oviposition Tests

Egg masses were found on all plant species tested, however, there were significantly more egg masses on silver buttonwood than the alternate choice, sorghum-sudan (t = 3.39; df = 29; P = 0.002) or lime (t = -2.83; df = 30; P = 0.008) (Table 3).

Three-Choice Oviposition Tests

The numbers of egg masses per plant were low. There were no significant differences among the three host plants, silver buttonwood, Tahiti lime and sour orange, although the highest mean number of egg masses occurred on silver buttonwood (F = 1.06; df = 2, 117; P = 0.3510) (Table 4). There were significantly more adults per plant on silver buttonwood compared with sour orange (F = 4.83; df = 2, 117; P = 0.0096) 8 days after the

TABLE 2. MEAN NUMBER OF EGG MASSES FROM 50 FEMALE *DIAPREPES ABBREVIATUS* IN A NO-CHOICE TEST.

	Host plant	Mean egg masses per plant (±SE)[1]
Test 1	*Manihot esculenta* (cassava)	4.75 ± 1.21 a
	Conocarpus erectus (silver buttonwood)	2.87 ± 1.25 ab
	Carica papaya (papaya)	0.50 ± 0.38 bc
	Xanthosoma sp. (malanga)	0.00 ± 0.00 c
Test 2	*Conocarpus erectus* (silver buttonwood)	1.75 ± 0.36 a
	Sorghum sudanense (sorghum-sudan)	1.25 ± 0.45 a
	Persea americana (avocado)	0.13 ± 0.12 b
	Chrysophyllum oliviforme (satinleaf)	0.00 ± 0.00 b
Test 3	*Conocarpus erectus* (silver buttonwood)	4.12 ± 1.24 a
	Solanum tuberosum (white potato)	2.25 ± 0.31 a
	Pennisetum purpureum (elephant grass)	2.25 ± 0.70 a
	Zea mays (sweetcorn)	1.75 ± 0.41 a

[1]Means within a column for each test followed by different letters are significantly different ($P < 0.05$).

adults were released into the screen house (Table 4). The number of female adults on silver buttonwood was significantly greater than on the other two plant species ($F = 6.79$; df = 2, 117; $P = 0.0016$), while the number of male adults was not ($F = 2.15$; df = 2, 117; $P = 0.1209$).

Multiple-Choice Oviposition Tests

Significantly more egg masses were found on the foliage of silver buttonwood compared with all other plants in the test ($F = 26.31$; df = 6, 326; $P = 0.0001$) (Table 5). No eggs were found on the foliage of malanga.

Overall, silver buttonwood appeared to be the preferred host for oviposition in all the choice tests. Although differences were not always significant, the highest mean numbers of egg masses per plant were on silver buttonwood in all tests but one. In the latter test, the second highest mean number of egg masses was found on silver buttonwood. Silver buttonwood is very common in nursery production, and in the landscape in southern Florida. The preference for silver buttonwood, however, did not preclude oviposition on other hosts. No choice tests were conducted without silver buttonwood but should be considered in future studies to help better understand host selection by adult weevils. In southern Florida ornamental nurseries, mixed species of plants are commonly planted within a row. Thus, female *D. abbreviatus* may lay eggs on the foliage of several species, despite the presence of a more preferred host, such as silver buttonwood.

There were other factors inherent in the choice bioassays that may have influenced the outcome. First, the egg-laying potential of the weevils was unknown because they were field collected. All weevils used in a given test were all collected at the same time, however, the choice tests were not conducted concurrently. Therefore, there could be differences in oviposition due to female age, condition, etc. Additionally, plant phenology could also influence the level of oviposition. Although plant phenology was not controlled for, all plants exhibited foliage that appeared suitable for oviposition. Lastly, all adult weevils collected from the field were caged and provided green buttonwood as a food source. Although the time the weevils here held before use was relatively short (24 h), feeding on green buttonwood prior to the test may have increased their preference for oviposition on silver buttonwood. Also, no tests were conducted without silver buttonwood. More tests are necessary to evaluate these influences as well as when no preferred hosts are available for oviposition.

TABLE 3. MEAN NUMBER OF EGG MASSES OF 50 FEMALE *DIAPREPES ABBREVIATUS* IN A TWO-CHOICE TEST.

	Host plant	Mean egg masses per plant (±SE)[1]
Test 1	*Conocarpus erectus* (silver buttonwood)	7.00 ± 1.22 a
	Sorghum sudanense (sorghum-sudan)	2.38 ± 0.65 b
Test 2	*Conocarpus erectus* (silver buttonwood)	0.88 ± 0.27 a
	Citrus aurantifolia (lime)	0.19 ± 0.03 b

[1]Means within a column for each test followed by different letters are significantly different ($P < 0.05$).

TABLE 4. THE MEAN NUMBER OF *DIAPREPES ABBREVIATUS* ADULTS AND EGG MASSES ON THREE PLANT SPECIES 8 DAYS AFTER RELEASE OF 500 ADULTS (1:1 MALE:FEMALE) INTO THE SCREEN HOUSE.

Plant species (common name)	Mean egg masses per plant[1] (±SE)	Mean adults per plant[1] (±SE)	Mean males per plant[1] (±SE)	Mean females per plant[1] (±SE)
Conocarpus erectus (silver buttonwood)	0.30 ± 0.12 a	1.53 ± 0.34 a	0.67 ± 0.20 a	0.90 ± 0.19 a
Citrus aurantifolia (Tahiti lime)	0.10 ± 0.05 a	0.60 ± 0.30 ab	0.33 ± 0.19 a	0.27 ± 0.13 b
Citrus sinensis (sour orange)	0.13 ± 0.13 a	0.17 ± 0.08 b	0.13 ± 0.06 a	0.03 ± 0.03 b

[1]Means within a column followed by different letters are significantly different ($P < 0.05$).

Larval Survival and Root Consumption

Survival of larvae and their effect on plant growth was examined on several commonly grown plant species in southern Florida. Both fresh and dry root weight (fresh: $t = 3.68$; df = 18; $P = 0.001$; dry: $t = 3.85$; df = 18; $P = 0.001$) and plant biomass (fresh: $t = 4.71$; df = 18; $P = 0.0002$; dry: $t = 3.58$; df = 18; $P = 0.002$) were significantly reduced on green bean as a result of larval feeding 2 months after infestation (Table 6). However, the measured traits of sweet corn were not altered (Table 6). Almost no larvae survived on the sweet corn but an average of 2.6 larvae survived per green bean plant (Table 7).

Larvae survived on silver buttonwood, lime, and sorghum-sudan 3 months after infestation (Table 7). Larvae did not survive on malanga or satinleaf (Table 7), and therefore, there was no effect on plant height, root weight and biomass of malanga or satinleaf (Table 6). The highest mean number of larvae survived on silver buttonwood. The fresh root weight and fresh biomass weight were significantly reduced in silver buttonwood plants infested with larvae with a 13.1 percent reduction in the dry biomass (root: $t = 3.30$; df = 20; $P = 0.003$; biomass: $t = 3.04$; df = 20; $P = 0.006$) (Table 6). On Tahiti lime, an average of 1.8 larvae per plant survived (Table 7), and both the fresh and dry root weight and biomass weight were significantly reduced (fresh root: $t = 3.33$; df = 42; $P = 0.001$; fresh biomass: $t = 8.02$; df = 42; $P = 0.0001$; dry root: $t = 3.07$; df = 42; $P = 0.004$; dry biomass: $t = 6.60$; df = 41; $P = 0.0001$) (Table 6). The net reduction of dry biomass was 42.9%. At the time of evaluation, the lime plants were dead or dying. An average of 5.5 larvae per plant survived on sorghum-sudan (Table 7). Both fresh and dry root weights (fresh: $t = 3.09$; df = 18; $P = 0.0063$; dry: $t = 3.83$; df = 18; $P = 0.003$) and fresh and dry biomass weights (fresh: $t = 2.71$; df = 18; $P = 0.014$; dry: $t = 2.71$; df = 10; $P = 0.02$) were significantly reduced as a result of larval feeding (Table 6). The overall reduction in biomass of the sorghum-sudan was 41.9%.

Larval survival was low on silver buttonwood, Tahiti lime and malanga 6 months after infestation and there were no significant differences among host plants ($F = 2.41$; df = 3, 28; $P = 0.08$). Nevertheless, silver buttonwood supported the highest mean number of larvae and these larvae had the highest weights (Table 7).

Regardless of the host plant infested, the number of larvae per plant that survived was low relative to the number of neonates initially used to inoculate (50). Neonates are highly mobile (Wolcott 1936), and some of them may actually leave the containers at the time of infestation. Neonates have been shown to move over the tops of containers as well as through holes in the bottoms of containers (Mannion, unpublished data). It is very difficult to prevent this movement. Mean weights of larvae varied with the infestation time and the host plant species. The highest mean larval weight 3 months after infestation was that of larvae feeding on silver buttonwood. Average

TABLE 5. MEAN NUMBERS OF EGG MASSES OF *DIAPREPES ABBREVIATUS* IN A MULTIPLE-HOST CHOICE TEST.

Plant species (common name)	Mean no. egg masses per plant (±SE)[1]
Conocarpus erectus (silver buttonwood)	1.66 ± 0.27 a
Zea mays (sweetcorn)	0.33 ± 0.06 b
Solanum tuberosum (white potato)	0.23 ± 0.07 b
Sorghum sudanense (sorghum-sudan)	0.18 ± 0.06 b
Pennisetum purpureum (elephant grass)	0.04 ± 0.03 b
Citrus aurantifolia (lime)	0.04 ± 0.03 b
Xanthosoma sp. (malanga)	0.00 ± 0.00 b

[1]Means within a column followed by different letters are significantly different ($P < 0.05$).

TABLE 6. THE EFFECT OF LARVAL FEEDING OF *DIAPREPES ABBREVIATUS* 2 MONTHS AFTER INFESTATION OF PLANTS INFESTED WITH 50 NEONATES.

Plant species (common name)	Treatment	n	Plant height (cm) (SE)	Fresh root weight (g)[1](SE)	Dry root weight (g)[1](SE)	Fresh biomass weight (g)[1](SE)	Dry biomass weight (g)[1](SE)	Percent dry biomass reduction
Phaseolus vulgaris (green bean)	Infested	10	n/a	0.233 b (0.14)	0.08 b (0.05)	11.30 b (1.80)	5.03 b (0.34)	55.1
	Not infested	10	n/a	2.49 a (0.60)	1.53 a (0.37)	45.90 a (7.12)	11.20 a (1.70)	
Zea mays (sweet corn)	Infested	10	15.27 a (0.41)	6.93 a (0.90)	5.51 a (0.54)	60.77 a (7.57)	36.56 a (2.67)	0.00
	Not infested	10	14.78 a (0.69)	5.82 a (0.67)	5.10 a (0.59)	50.79 a (4.93)	34.57 a (3.16)	
Conocarpus erectus (silver buttonwood)	Infested	6	115.2 a (3.19)	82.85 b (19.90)	25.32 b (4.21)	362.32 b (27.25)	110.13 a (6.81)	13.1
	Not infested	5	117.8 a (11.27)	141.36 a (4.96)	33.70 a (1.60)	453.96 a (8.27)	126.72 a (4.54)	
Citrus aurantifolia (Tahiti lime)	Infested	8	57.3 a (3.80)	25.74 b (8.26)	12.53 a (1.91)	82.07 b (9.14)	57.91 b (6.32)	42.9
	Not infested	8	59.8 a (3.71)	121.79 a (31.79)	29.14 a (7.55)	281.71 a (39.03)	101.39 a (12.28)	
Xanthosoma sp. (malanga)	Infested	8	62.88 a (5.38)	234.8 a (21.25)	30.38 a (4.05)	416.65 a (55.78)	44.51 a (6.65)	0.0
	Not infested	8	73.13 a (2.95)	202.5 a (20.27)	20.64 a (2.42)	387.29 a (42.37)	32.48 a (3.71)	
Chrysophyllum oliviforme (satinleaf)	Infested	5	120.6 a (7.20)	203.82 a (40.95)	114.14 a (22.82)	369.02 a (63.54)	207.00 a (35.19)	3.1
	Not infested	5	115.8 a (6.80)	253.90 a (52.64)	116.48 a (23.20)	427.68 a (59.49)	213.52 a (29.82)	
Sorghum sudanense (sorghum-sudan)	Infested	10	232.8 a (4.50)	34.76 b (5.38)	18.48 b (1.14)	355.99 b (28.59)	122.44 b (7.27)	41.9
	Not infested	10	236.4 a (5.80)	60.71 a (6.44)	25.00 a (2.32)	472.10 a (31.97)	210.69 a (16.92)	

[1]Means within a column for each plant species followed by different letters are significantly different ($P < 0.05$).

TABLE 7. LARVAL SURVIVAL AND LARVAL WEIGHTS OF *DIAPREPES ABBREVIATUS* ON DIFFERENT HOST PLANTS EACH INFESTED WITH 50 NEONATES.

	2 Months after infestation		3 Months after infestation		6 Months after infestation	
Plant species	Mean larvae (SE)	Mean larval weight (g) (SE)	Mean larvae[1] (SE)	Mean larval weight (g)[1] (SE)	Mean larvae[1] (SE)	Mean larval weight (g)[1] (SE)
Phaseolus vulgaris (green bean)	2.6 (0.37)	0.20 (0.05)	—	—	—	—
Zea mays (sweet corn)	0.2 (0.20)	0.02 (0.02)	—	—	—	—
Conocarpus erectus (silver buttonwood)	—	—	7.5 (0.71) a	0.13 (0.01) a	1.217 (0.65) a	0.130 (0.05) a
Citrus aurantifolia (lime)	—	—	1.8 (0.61) b	0.09 (0.02) a	0.13 (0.13) a	0.03 (0.03) a
Xanthosoma sp. (malanga)	—	—	0.0 c	0.0 b	0.13 (0.13) a	0.02 (0.17) a
Chrysophyllum oliviforme (satinleaf)	—	—	0.0 c	0.0 b	—	—
Sorghum sudanense (sorghum-sudan)	—	—	5.5 (1.16) a	0.07 (0.01) a	—	—

[1]Means within a column followed by different letters are significantly different ($P < 0.05$).

weights of larvae feeding on the other hosts 3 months after infestation were relatively low.

Numerous plant hosts are suitable as a food source and for oviposition by adult *D. abbreviatus* as well as supporting larvae. In our study, silver buttonwood, a common landscape plant in south Florida, was generally preferred. More larvae survived and more egg masses were found on this host plant. However, it is important to note that in the absence of silver buttonwood, other plant species still provide suitable sites for oviposition and larval survival. Schroeder et al. (1979) found 9 species of ornamental plants and one native plant species other than citrus and sugarcane to be suitable for larval development. Simpson et al. (1996) identified nine plant species that support oviposition and larval development. More than 40 plant species were associated with larval feeding. The host plants identified as having some association with *D. abbreviatus* are diverse belonging to 59 plant families. Eggs may be present without feeding adults and larvae may be present without evidence of oviposition. The survival of larvae and subsequent damage from root feeding for most plant host is not known. Dispersion of this pest is likely by the movement of plant material infested with any of the life stages of *D. abbreviatus*. Currently, this pest is considered a regulatory risk and any plant associated with any life stage of *D. abbreviatus* is considered a regulatory host. Growers in known infested counties are required to follow strict guidelines of treatments, which are time-consuming, expensive, and disruptive to natural enemies, before shipping plant material to non-infested areas.

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OVIPOSITION BY *METAMASIUS HEMIPTERUS SERICEUS* (COLEOPTERA: DRYOPHTHORIDAE: RHYNCHOPHORINAE)

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ABSTRACT

Metamasius hemipterus sericeus (Olivier) is a widely distributed weevil in Central and South America, as well as the West Indies. It was introduced into Florida, Miami-Dade County, in 1984. This insect generally is regarded as a secondary pest of sugarcane, bananas, palms and several other tropical plants grown as ornamentals. Larvae bore into stems and petioles, thus weakening the plant and providing a pathway for penetration by fungi or other pests. In addition to investigating the biology, this study was conducted to gather basic information to help optimize culturing efforts for large numbers of *M. h. sericeus* to be used for mass rearing of potential biological control organisms. After pairing males and females, it took an average of 27.0 days for females to begin oviposition. The oviposition period lasted 56.8 days. Females lived 142.3 days and laid an average of 51.6 eggs. Mean generation time was 63 days. Mean egg production during the oviposition period was 1.1 eggs/day. Egg eclosion averaged 81.3% during the oviposition period.

Key Words: Dryophthoridae, Rhynchophorinae, silky cane weevil, fertility, fecundity

RESUMEN

El picudo rayado *Metamasius hemipterus sericeus* (L.) esta ampliamente distribuido en Centro y Suramerica, así como también en las Indias Occidentales. Fué introducido en el condado de Miami-Dade, Florida en 1984. Se le considera una plaga de segunda importancia en caña de azucar, banano, palmas y en varias plantas ornamentales tropicales. La larva perfora los tallos y peciolos, debilitando la planta y brindando un puerto de entrada a hongos y otras plagas. Ademas de investigar la biologia basica durante este estudio, hemos generado información con el fin de ayudar a la optimizacion de metodos de crianza masiva de *M. h. sericeus*. Las hembras comienzan a ovipositar en un promedio de 27 días despues de aparear con los machos. El periodo de oviposición dura 56.8 días. Las hembras viven 142.3 días y ovipositan un promedio de 51.6 huevos. El promedio de produccion diaria de huevos por hembra fué de 1.1. El porcentaje promedio de eclosión de huevos durante el período de oviposición fué de 81.3%.

Translation provided by author.

Metamasius hemipterus sericeus (Olivier), a weevil that is widely distributed in Central and South America, as well as the West Indies, was introduced into Florida and reported there for the first time in Miami-Dade County in 1984 (Woodruff & Baranowski 1985). Generally it is regarded as a secondary pest of sugarcane, bananas, palms and many other tropical plants grown as ornamentals. The larvae bore into stems and petioles, thus weakening the plant and providing a pathway for penetration by fungi or other pests.

According to the literature, *M. h. sericeus* adults live for 60 days and females lay 500 eggs (Castrillon & Herrera 1986). Females are attracted to and oviposit in damaged or stressed host tissues (Giblin-Davis et al. 1994). Eggs hatch in about 4 days and larvae begin to feed. In sugarcane, larvae feed in the pith, sometimes boring into healthy tissue. Larval tunneling in palms starts in the petioles, crown, or stem, usually in wounds, and extend into healthy tissue. After about 7 weeks, larvae construct a fibrous pupal case. After 10 days, pupae transform to adults, which may immediately break free of the cocoon, or may remain within the cocoon until conditions are favorable for emergence. The mean generation time is 63 days (Woodruff & Baranowski 1985). Adults of *M. h. sericeus* are free living, and often are found on or within banana pseudostems, palm fronds, sugarcane sheaths, and leaf litter.

Metamasius hemipterus sericeus poses a significant threat to the economic establishment of the

sugarcane cultivar 'CP-85-1382' (Sosa et al. 1997) and nursery grown palms. The expense of control with traditional insecticides or with biopesticides can increase production costs substantially. Biological control, however, offers the potential of long term, relatively inexpensive control of *M. h. sericeus*. A logical candidate for classical biological control of *M. h. sericeus* in Florida is *Lixophaga sphenophori* (Villeneuve), a tachinid parasitoid used to manage a sugarcane weevil species, *Rhabdoscelus obscurus* (Boisduval), in New Guinea (Waggy & Beardsley 1972) and Hawaii that is closely related to *M. h. sericeus*. Assuming *L. sphenophori* will parasitize *M. h. sericeus*, large numbers of host larvae need to be reared.

This study was conducted to gather basic information to help optimize culturing efforts for large numbers of *M. h. sericeus* to be used for mass-rearing *L. sphenophori* or other potential biological control organisms. In addition, we are studying the biology of this pest as part of a long-range objective leading to management.

Materials and Methods

Metamasius hemipterus sericeus adults were collected December 1998-March 1999 in Broward Co., FL using optimized pheromone bucket traps described by Giblin-Davis et al. (1996). As they became available, trapped weevils were placed in 68-l plastic storage tubs (usually 30 or more weevils were caged per tub). Each tub was provisioned with 3 kg of sugarcane stem cut into 0.2 m length pieces, and covered with screening to provide ventilation and prevent weevil escape. Tubs were left outdoors in a location sheltered from rainfall and sunlight. *Metamasius hemipterus sericeus* cocoons were periodically collected from sugarcane stem pieces and held in an incubator set at 27°C until adults emerged. Upon emergence, adults were sexed by the presence of tufts of hair on the apical segment of the abdomen (males) or the absence of these tufts (females) (Vaurie 1966). Males and females were paired and placed in 200 ml cups covered by lids with a 4.5 cm diam. hole covered by aluminum screen (14 mm opening). To prepare an ovipositional substrate, sugarcane stems were peeled and thinly sliced (1.4-1.8 mm thick). Preliminary observations demonstrated that eggs laid in thin slices could be removed easily and counted. Cane slices much thicker than this resulted in poor or inaccurate egg recovery. Cane slices (1 per oviposition cup) were placed over the screened cup opening and covered with a water-moistened piece of filter paper. Cups were inverted and placed outdoors [mean low 24.5 ± 1.4°C (SD); mean high 29.4 ± 1.5°C (SD); range 20-33°C] in a location protected from sunlight and rain. Cane slices were replaced daily, and old slices were dissected for eggs. Males were removed from oviposition cups the first day after eggs were found on cane slices. Eggs were carefully removed from the cane and individually placed in petri dishes (15 × 100 mm) lined with water-moistened filter paper. Individual confinement was determined to be necessary as a preventative measure against possible larval cannibalism. Petri dishes containing eggs were placed in an incubator set at 27°C and checked daily for eclosion. A total of 29 cups were monitored daily for oviposition, and for female longevity. Measurements were made on 14 eggs to determine dimensions.

Descriptive statistics (means, standard deviation and range) were calculated and used to help describe observed parameters. Linear regression was used to determine if eclosion changed through time.

Results and Discussion

Eggs are oblong and measure 1.31 mm (± 0.08 [SD]; range = 1.17-1.44 mm) in length and 0.44 mm (± 0.05 [SD]; range = 0.39-0.51 mm) in width. Twenty-two of the 29 weevils (76%) observed laid eggs. After pairing newly emerged males with females, it took an average of 27.0 d (± 11.3 [SD]; range = 7-95 days) for females to begin laying eggs. Of the females that laid eggs, the oviposition period lasted an average of 59.4 d (± 5.7 [SD]; range = 12-128 days). Females lived on average 142.3 d (± 7.8 [SD]; range = 40-204 days) and laid an average of 51.6 eggs (± 1.4 [SD]; range = 0-192 eggs). Mean egg production per female during the oviposition period was 1.1 eggs/day (± 0.02 [SD]; range = 0.08-3.3 eggs/days).

Fecundity observed in this study was considerably less than that reported by Castrillon & Herrera (1986) who observed up to 500 eggs laid per *M. h. sericeus* female. However, it is unclear how these data were collected. Observed fecundity between this work and that of Castrillon & Herrera (1986) may be due to differences in ovipositional substrates offered to weevils. For example, Giblin-Davis et al. (1989) using pineapple as an oviposition medium estimated fecundity of *R. cruentatus* to be substantially less than fecundity observed when weevils were allowed to oviposit in apples (Weissling & Giblin-Davis 1994). In preliminary experiments, *M. h. sericeus* females were offered many ovipositional substrates, including apple slices, banana stem, agar, and various arrangements of sugarcane chunks and slices. Resulting oviposition was poor and results were variable. However, females readily oviposited in thin sugarcane slices (1.4-1.8 mm thick) and the eggs were easy to locate and remove from the tissue.

Newly-emerged *M. h. sericeus* females laid few if any eggs during the first two-weeks of confinement with males. The greatest number of eggs were laid during the third through eleventh weeks, after which egg production generally declined (Fig. 1). There was a slight increase in oviposition 19

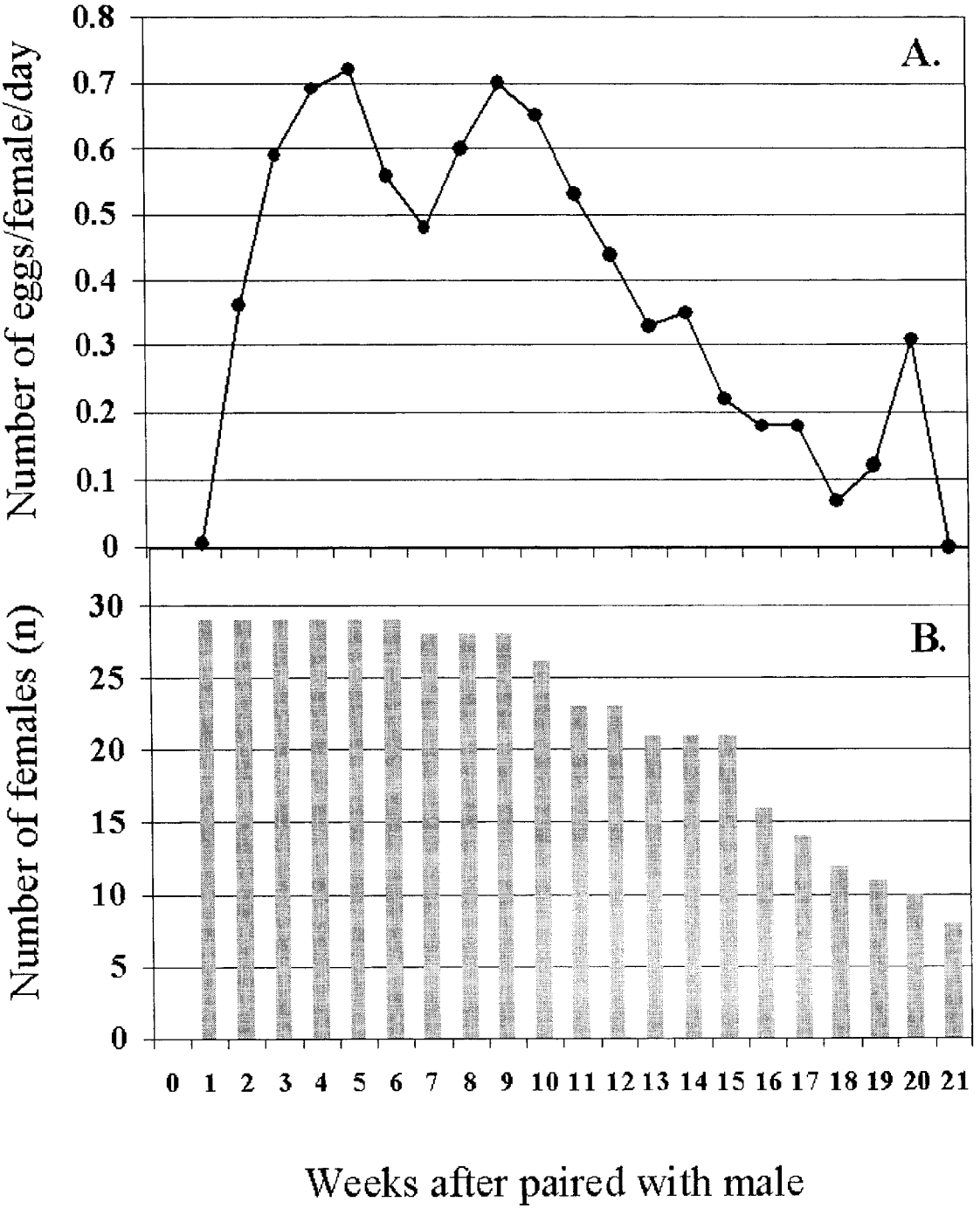

Fig. 1. Mean weekly egg production by *M. h. sericeus* females (A) and number of females observed (B) confined individually on sugarcane slices.

and 20 weeks after pairing. A similar trend in late-oviposition period productivity was observed with *R. cruentatus* (Weissling & Giblin-Davis 1994). Reasons are unclear for this observation.

Fertility of eggs laid by *M. h. sericeus* varied through time but remained at a fairly high level during the 15 weeks of observation (Fig. 2). Regression analysis over time revealed no clear

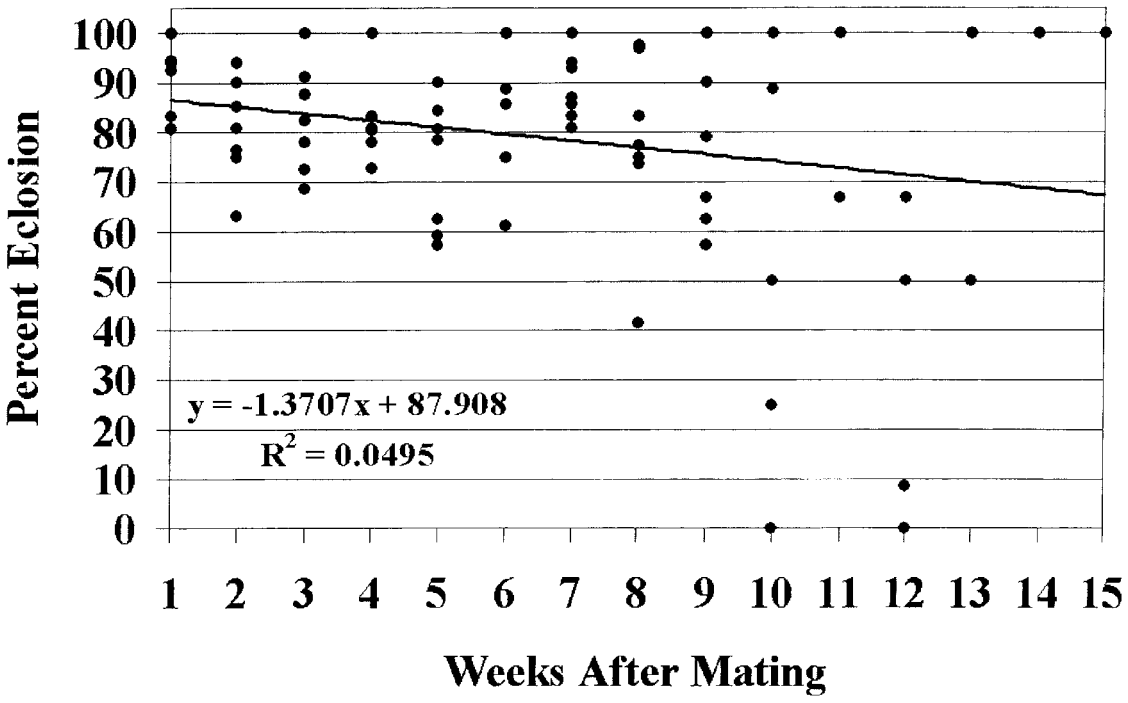

Fig. 2. Percent eclosion of eggs produced by *M. h. sericeus* females through time.

linear trend ($R^2 < 0.05$). Throughout the experimental period, eclosion averaged 81.3%. These results indicate that mating during the preoviposition period resulted in the transfer of adequate quantities of sperm to fertilize eggs without subsequent mating. In contrast, fertility of *R. cruentatus* eggs declined to zero 9 weeks after mating (Weissling and Giblin-Davis 1994).

The observed mean fecundity of *M. h. sericeus*, although lower than that reported by Castrillon & Herrera (1986), is at levels high enough to make mass rearing for the production of parasitoids feasible. Based on specifics of this study, weevil culture can be optimized by providing females with thin sugarcane slices for oviposition. These slices could then be transferred to large sugarcane pieces for larval feeding.

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EFFECTS OF INSECTICIDES ON *ORIUS INSIDIOSUS* (HEMIPTERA: ANTHOCORIDAE), MEASURED BY FIELD, GREENHOUSE AND PETRI DISH BIOASSAYS

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ABSTRACT

Orius insidiosus (Say) is an important predator of several economic pests in cotton. Laboratory-reared males, females and third instar nymphs were exposed to residues of nine insecticides applied to cotton plants in the field, in potted plants in the greenhouse and glass Petri dishes in the laboratory. Insects were exposed for 24-hours and then removed to determine mortality. Insecticides tested were spinosad, indoxacarb, imidacloprid, tebufenozide, methoxyfenozide, abamectin, emamectin benzoate, fipronil and λ-cyhalothrin. Differences were observed in mortality as measured by different methods. Spinosad, imidacloprid and indoxacarb induced significantly higher mortality with treated Petri dishes than on treated cotton plants in the field or greenhouse. No differences in mortality were observed between methods with fipronil or λ-cyhalothrin, and in only one instance with abamectin. Spinosad was not toxic in the field or greenhouse bioassays, but was highly toxic in the Petri dish bioassay. Imidacloprid was moderately toxic in the field and greenhouse, but was highly toxic in the Petri dish bioassay. Indoxacarb had variable toxicity, with low to moderate toxicity in the field and greenhouse, and high toxicity in the Petri dish bioassay. It is apparent that multiple testing methods should be used in evaluating the effects of pesticides on beneficial arthropods.

Key Words: insidious flower bug, pesticides, mortality

RESUMEN

Orius insidiosus (Say) es un depredador importante de diferentes plagas económicas en el algodón. Machos criados en el laboratorio, hembras y ninfas del tercer estádio fueron expuestos a residuos de nueve insecticidas aplicados a plantas de algodón en el campo, en plantas en masetas en el invernadero, y en platos de Petri de vidrio en el laboratorio. Los insectos fueron expuestos por 24-horas y después sacados para determinar la mortalidad. Los insecticidas probados fueron spinosad, indoxacarb, imidacloprid, tebufenozide, methoxyfenozide, abamectin, emamectin benzoate, fipronil λ-cyhalothrin. Se observaron diferencias en la mortalidad medida por métodos diferentes. Los spinosad, imidacloprid e indoxacarb inducian una mortalidad significativamente más alta en los platos tratados en los Petri tratados que en las plantas de algodón en el campo y en el invernadero. Ninguna diferencia en la mortalidad fué observada entre los métodos con fipronil λ-cyhalothrin, y solamente en una ocasión con abamectin. El spinosad no fue tóxico en los bioensayos del campo o del invernadero, pero fué altamente tóxico en el bioensayo en el plato Petri. Imidacloprid fué moderadamente tóxico en el campo y en el invernadero, pero fué altamente tóxico en el bioensayo en el plato de Petri. Indoxacarb tenia una tóxicidad variable, con una tóxicidad de baja a moderada en el campo y en el invernadero, y altamente tóxico en el bioensayo en el plato de Petri. Es evidente que se debe usar métodos de pruebas multiples para evaluar los efectos de pesticidas en artrópodos beneficos.

An increasing number of scientists are evaluating the toxicity of new pesticide chemistries on beneficial arthropods. Although a considerable number of studies have been published, it is sometimes difficult to compare results among researchers. The variety of methods used in bioassays is as varied as the number of individuals conducting the work. Scientists have used direct topical applications of the pesticide to the insect (Yu 1988; De Cock et al. 1996; Trisyono et al. 2000) or injected the insect with insecticide (Yu 1988), or fed treated prey (De Cock et al. 1996; Trisyono et al. 2000; Elzen 2001). These methods insure that a specific insecticide dose makes contact with the insect, either topically or internally, and will give an accurate indication of the actual toxicity of the pesticide to the insect in question. It is likely that if the insect species survives the topical or injection application, it will also survive any exposure in the field. However, the reverse

may not always be true. High mortality resulting from a topical or injection bioassay may not be related to mortality observed under field conditions.

Researchers commonly use an inert substrate such as glass vials, Petri dishes or slides to test the toxicity of various insecticide residues to predatory or parasitic insects (Plapp & Bull 1978; Mizell & Schiffhauer 1990; Bayoun et al. 1995; Oetting & Latimer 1995; De Cock et al. 1996) or may use treated plastic cups (Mizell & Schiffhauer 1990). There are several possible errors that could occur in using treated substances such as glass or plastic to evaluate the toxicity of any insecticide to an insect. While data from these bioassays will give an indication of the toxicity of a pesticide to an insect, relating this toxicity to that which may be encountered in the field is difficult. The activity of a pesticide may be affected by the substrate upon which it is deposited (Cogburn 1972; White 1982). Jain and Yadav (1989) found that some insecticides persisted much longer when applied to a plastic substrate as compared with glass or painted wood.

Potentially more realistic testing methods use treated excised leaves (Samsoe-Petersen 1985; Oetting & Latimer 1995; Jones et al. 1997; Elzen & Elzen 1999), or treated potted plants grown in the greenhouse (Brown & Shanks 1976; Pietrantonio & Benedict 1999). These methods should provide a more realistic picture of actual toxicity from contact with residues on a natural substrate. Environmental effects (e.g., solar radiation or insect movement within the plant canopy) which may effect actual toxicity are not addressed.

Numerous researchers have evaluated insecticide effects on beneficial arthropods by making evaluations in the field from treated field plots (Brown & Shanks 1976; Stoltz & Stern 1978; Young et al. 1997; Simmons & Jackson 2000). Generally, the results from field studies express toxicity as the resulting presence or absence of the insect from a treated plot in comparison with an untreated plot or with pretreatment counts. These data are often taken within a few days to a week after treatment, depending on the researcher and the experimental design. In many instances the studies were designed to evaluate mortality induced in the target pest, with beneficial arthropod counts being made as a secondary goal to the study. The test plots in this latter case are not designed to accurately evaluate the induced mortality in the beneficial arthropods naturally present in the study.

In evaluating the effects of pesticides on any insect, the method used may have an effect on the final results. Confounding factors include solar radiation, rainfall, substrate treated, temperature, etc. Under field conditions, the effectiveness of a properly-applied insecticide may be diminished by high temperatures, sunlight and rainfall events. Similarly, the same tests may underestimate mortality caused by those insecticides that are systemic in the plant tissue, particularly on plant feeding insects. Therefore, it would be important to compare these effects as measured through various methodologies.

Materials and Methods

Orius insidiosus (Say) were collected from host plants (crimson clover, vetch and corn) early in the season of each year and used to start a lab colony maintained on green bean pods and *Helicoverpa zea* (Boddie) eggs. *H. zea* pupae were obtained from a colony maintained at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, AR. Once adult moths emerged, they were placed in aquariums covered with a layer of cheesecloth onto which the females could oviposit. Wild adult moths were also collected and added to the colony during the growing season when they were abundant. *O. insidiosus* were reared at a photoperiod of 14:10 (L:D) at 25°C in an illuminated incubator (Precision Scientific® model 818, Winchester, VA). Green bean pods were not only a source of food and moisture, but also served as a substrate into which females would readily oviposit. Green beans and *H. zea* eggs were replaced daily. Pods with *O. insidiosus* eggs were placed into separate containers to allow nymphs to hatch. Fresh bean pods and *H. zea* eggs were provided to nymphs as well.

Field Plots

Plots of SureGrow 125 cotton were planted at the University of Arkansas Northeast Research and Extension Center, Keiser, AR during the growing seasons of 2000 and 2001. Fertility and weed control recommendations outlined by the University of Arkansas Cooperative Extension Service were followed (Baldwin et al. 2001). No insecticides were applied to plots with the exception of the insecticide treatments outlined in this study (Table 1). Also, no in-furrow insecticides were applied at planting to insure insecticide-free plants. Plots were 4 rows by 7.6-m long arranged in a RCB design with 4 replications. Insecticides were applied using a CO_2 powered backpack sprayer. The sprayer was calibrated to deliver 10 gpa at a pressure of 40 psi through 2-TX8 hollow-cone nozzles per row. Water alone was applied to the untreated control plots. Only the center 2 rows of each plot were treated to give a buffer of 2 rows between each pair of treated rows. Treatments were applied early in the morning, just after sunrise, when wind conditions were negligible to avoid spray drift. The spray boom was cleaned between each treatment by rinsing with a water and bleach solution, followed by water.

O. insidiosus were caged on plants as soon as sprays had dried (approximately 1-h after applica-

TABLE 1. PERCENT MORTALITY IN *O. INSIDIOSUS* MALES MEASURED BY THREE METHODS IN 2000.

Insecticide	Rate kg ai/ha	Field[1]	Greenhouse[1]	Petri dish[1]
Untreated		21.3 cAB	12.5 dB	26.3 cA
Spinosad	0.09	18.8 cB	13.8 dB	98.8 aA
Spinosad	0.199	23.8 cB	21.3 dB	92.5 aA
Indoxacarb	0.078	25.0 cB	20.0 dB	100.0 aA
Indoxacarb	0.123	21.3 cB	16.3 dB	100.0 aA
Imidacloprid	0.027	42.5 bB	46.3 bcB	100.0 aA
Imidacloprid	0.053	53.8 bB	53.3 bB	100.0 aA
Methoxyfenozide	0.28	23.8 cA	18.8 dA	25.0 cA
Methoxyfenozide	0.84	27.5 cAB	36.3 cA	18.3 cB
Tebufenozide	0.14	23.8 cA	21.3 cA	28.8 cA
Tebufenozide	0.28	23.8 cA	23.8 cA	20.0 cA
Emamectin benzoate	0.005	100.0 aA	100.0 aA	91.3 aA
Emamectin benzoate	0.01	100.0 aA	100.0 aA	78.8 bB
Abamectin	0.01	100.0 aA	100.0 aA	100.0 aA
Abamectin	0.02	98.8 aA	100.0 aA	100.0 aA
Fipronil	0.042	100.0 aA	100.0 aA	100.0 aA
Fipronil	0.056	100.0 aA	100.0 aA	100.0 aA
λ-cyhalothrin	0.014	100.0 aA	100.0 aA	100.0 aA
λ-cyhalothrin	0.028	100.0 aA	100.0 aA	100.0 aA

Means within a column followed by the same lower case letter and means within a row followed by the same upper case letter do not significantly differ ($P \leq 0.05$, LSD).

[1]A total of 80 individuals were used per treatment.

tion). Cages were placed on the fourth leaf down from the plant's terminal. Insects were caged on the plants for 24 h and then removed to evaluate mortality. Cages were constructed from 11.5 cm hair clips that were bent to fit around 6 cm diameter polystyrene Petri dishes. Each cage was constructed of either 2 Petri dish bases or 2 Petri dish tops so that the edges would meet forming an enclosure. Strips of foam were glued to the edges of each dish so that a seal would form when the cage was closed. A hole 3.2-cm in diameter was cut in each side of the cage and a piece of organdy cloth was glued over the opening to allow for air flow through the cage. Males, females and third instar nymphs were evaluated separately to determine the effects on gender and insect stage. Data were arcsine transformed and means from all bioassays were subjected to analysis of variance and separated by least significant difference test (LSD, $P \leq 0.05$). Detransformed means are reported.

Greenhouse

SureGrow 125 cotton was grown in pots in the greenhouse at the University of Arkansas Northeast Research and Extension Center, Keiser, AR. Potted plants were treated in a DeVries model SB8 spray chamber. The chamber was calibrated to deliver 11.5 gpa through a single TX8 hollowcone nozzle. Potted plants were treated individually with insecticide and then placed back into the greenhouse. The spray chamber nozzle was cleaned between each treatment by rinsing with a water and bleach solution, followed by pure water. *O. insidiosus* were caged on plants as soon as sprays had dried (approximately 1 h after application). Insects were caged on the plants for 24 h and then removed to evaluate mortality. Cages were the same as those used in the field study (20 per replicate). Males, females and third instar nymphs were evaluated separately to determine the effects on gender and insect stage. Data were arcsine transformed and means from all bioassays were subjected to analysis of variance and separated by least significant difference test (LSD, $P \leq 0.05$). Detransformed means are reported.

Laboratory

Glass Petri dishes 6-cm in diameter were treated with the insecticides listed in Table 1. Dishes were treated in the same spray chamber as the potted plants at the same rate (20 dishes per replicate, 4 replications). Individual *O. insidiosus* were placed in each dish as soon as sprays had dried (approximately 1-h after application), which was then covered with a piece of parafilm to keep insects from escaping. Mortality was checked after 24 h. Data were arcsine transformed and means from all bioassays were subjected to analysis of variance and separated by least significant difference test (LSD, $P \leq 0.05$). Detransformed means are reported.

Results and Discussion

Abamectin, emamectin benzoate, fipronil and λ-cyhalothrin were consistently the most toxic of the tested insecticides to *O. insidiosus* as measured by all three methods during 2000 (Tables 1-3) and 2001 (Tables 4-6). Mortality from λ-cyhalothrin ranged from 95% to 100%, fipronil 77% to 100%, emamectin benzoate 61% to 100% and abamectin 56.3% to 100%. No differences in mortality were observed for any of the three methods with fipronil or λ-cyhalothrin.

In all instances, mortality induced by abamectin and emamectin benzoate was significantly higher than that in the untreated control. In three instances, the mortality measured after treatment with these two products using the Petri dish bioassay was significantly lower than that in the field and greenhouse bioassays. In two instances mortality measured in the Petri dish bioassay was significantly higher than that in the field bioassay. In all other instances mortality was not significantly different among methods with these two pesticides.

Mortality induced by tebufenozide and methoxyfenozide was not significantly different from that of the untreated control when measured by field or Petri dish bioassays. However, in a few instances, mortality was significantly higher with these two insecticides when measured by the greenhouse bioassay.

Differences in mortality measured between methods was most consistent with spinosad, imidacloprid and indoxacarb. In every instance, mortality was much higher in the Petri dish bioassay compared with both the field and greenhouse bioassays. This was most pronounced with spinosad. While no significant mortality was observed in the field and greenhouse bioassays, mortality was very high in the Petri dish bioassay with spinosad. Mortality was also quite high in the Petri dish bioassay with imidacloprid and indoxacarb, but the difference was not as great because mortality was approximately 50% in the field and greenhouse bioassays.

Overall, there were few differences in mortality as measured by the field and greenhouse bioassays. The majority of significant differences were between these two plant bioassays and the Petri dish bioassay. The field and greenhouse bioassays would be expected to be similar in the fact that both use the same substrate, treated cotton leaves. The only differences between the two would be environmental conditions (e.g., solar radiation, temperature, relative humidity).

Croft (1990) defines that mortality or sublethal effects of pesticides occur through three avenues: 1) direct contact with the insecticide, 2) residual uptake (contacting pesticide residues on another surface), and 3) food chain uptake (consuming prey or host plants containing the pesticide). In this study, obviously *O. insidiosus* could only take up pesticide through the residual uptake avenue in the Petri dish bioassay. However, because of this insect's omnivorous habit, the possible uptake on treated plants used in the field and green-

Table 2. Percent mortality in *O. insidiosus* females measured by three methods in 2000.

Insecticide	Rate kg ai/ha	Field[1]	Greenhouse[1]	Petri dish[1]
Untreated		15.0 dA	8.8 eA	11.3 dA
Spinosad	0.09	20.0 dB	15.0 eB	92.5 aA
Spinosad	0.199	17.5 dB	21.3 deB	100.0 aA
Indoxacarb	0.078	18.8 dB	16.3 deB	81.3 bcA
Indoxacarb	0.123	28.8 cdB	18.8 deB	92.5 aA
Imidacloprid	0.027	36.3 cB	36.3 bcB	100.0 aA
Imidacloprid	0.053	52.5 bB	46.3 bB	100.0 aA
Methoxyfenozide	0.28	20.0 dA	21.3 deA	23.8 dA
Methoxyfenozide	0.84	22.5 dA	17.5 deA	22.0 dA
Tebufenozide	0.14	13.8 dA	15.0 eA	18.8 dA
Tebufenozide	0.28	27.5 cdA	28.8 cdA	21.3 dA
Emamectin benzoate	0.005	100.0 aA	100.0 aA	68.8 cB
Emamectin benzoate	0.01	100.0 aA	100.0 aA	90.0 aA
Abamectin	0.01	100.0 aA	100.0 aA	81.3 bcB
Abamectin	0.02	97.5 aA	100.0 aA	87.5 abA
Fipronil	0.042	92.5 aA	97.5 aA	92.5 aA
Fipronil	0.056	96.3 aA	98.8 aA	96.3 aA
λ-cyhalothrin	0.014	95.0 aA	100.0 aA	100.0 aA
λ-cyhalothrin	0.028	100.0 aA	100.0 aA	100.0 aA

Means within a column followed by the same lower case letter and means within a row followed by the same upper case letter do not significantly differ ($P \leq 0.05$, LSD).

[1]A total of 80 individuals were used per treatment.

TABLE 3. PERCENT MORTALITY IN *O. insidiosus* NYMPHS MEASURED BY THREE METHODS IN 2000.

Insecticide	Rate kg ai/ha	Field[1]	Greenhouse[1]	Petri dish[1]
Untreated		20.0 dA	12.5 deA	25.0 bcA
Spinosad	0.09	21.3 dB	25.0 cdB	91.3 aA
Spinosad	0.199	30.0 dB	21.3 cdeB	95.0 aA
Indoxacarb	0.078	17.5 dB	16.3 deB	91.3 aA
Indoxacarb	0.123	23.8 dB	25.0 cdB	100.0 aA
Imidacloprid	0.027	23.8 dB	26.3 cdB	100.0 aA
Imidacloprid	0.053	52.5 cB	51.3 bB	100.0 aA
Methoxyfenozide	0.28	26.3 dA	32.5 cA	27.5 bcA
Methoxyfenozide	0.84	18.8 dA	16.3 deA	16.5 bcA
Tebufenozide	0.14	13.8 dB	8.8 eB	32.5 bA
Tebufenozide	0.28	20.0 dA	26.3 cdA	15.0 cA
Emamectin benzoate	0.005	100.0 aA	100.0 aA	93.8 aA
Emamectin benzoate	0.01	100.0 aA	100.0 aA	97.5 aA
Abamectin	0.01	100.0 aA	100.0 aA	98.8 aA
Abamectin	0.02	97.5 aA	98.8 aA	100.0 aA
Fipronil	0.042	100.0 aA	100.0 aA	100.0 aA
Fipronil	0.056	80.0 bB	95.0 aA	100.0 aA
λ-cyhalothrin	0.014	100.0 aA	100.0 aA	100.0 aA
λ-cyhalothrin	0.028	100.0 aA	100.0 aA	100.0 aA

Means within a column followed by the same lower case letter and means within a row followed by the same upper case letter do not significantly differ ($P \leq 0.05$, LSD).

[1]A total of 80 individuals were used per treatment.

house bioassays could be through residual uptake and/or food chain uptake. With uptake possibly occurring through two avenues, one would expect mortality to be higher in the greenhouse and field bioassays as compared with the Petri dish bioassay. Although this was not true in the majority of cases in this study. Imidacloprid, indoxacarb and spinosad had much higher mortality in the

TABLE 4. PERCENT MORTALITY IN *O. INSIDIOSUS* MALES MEASURED BY THREE METHODS IN 2001.

Insecticide	Rate kg ai/ha	Field[1]	Greenhouse[1]	Petri dish[1]
Untreated		12.5 dA	7.5 eA	16.3 cA
Spinosad	0.09	13.8 dB	15.0 eB	78.8 bA
Spinosad	0.199	27.5 dB	33.8 cB	80.0 abA
Indoxacarb	0.078	52.5 cB	67.5 bB	91.3 abA
Indoxacarb	0.123	47.5 cB	53.3 bcB	97.5 abA
Imidacloprid	0.027	48.8 cB	31.3 cdB	97.5 abA
Imidacloprid	0.053	51.3 cB	47.5 bcB	96.3 abA
Methoxyfenozide	0.28	7.5 dA	12.5 eA	11.3 cA
Methoxyfenozide	0.84	12.5 dA	10.0 eA	8.8 cA
Tebufenozide	0.14	8.8 dA	7.5 eA	8.8 cA
Tebufenozide	0.28	18.8 dA	22.5 deA	10.0 cA
Emamectin benzoate	0.005	82.5 abA	96.3 aA	97.5 abA
Emamectin benzoate	0.01	68.8 bcB	90.0 aA	97.5 abA
Abamectin	0.01	88.8 abA	96.3 aA	78.8 bA
Abamectin	0.02	90.0 aA	95.0 aA	86.3 abA
Fipronil	0.042	91.3 aA	90.0 aA	97.5 abA
Fipronil	0.056	93.8 aA	91.3 aA	98.8 abA
λ-cyhalothrin	0.014	100.0 aA	100.0 aA	100.0 aA
λ-cyhalothrin	0.028	100.0 aA	100.0 aA	100.0 aA

Means within a column followed by the same lower case letter and means within a row followed by the same upper case letter do not significantly differ ($P \leq 0.05$, LSD).

[1]A total of 80 individuals were used per treatment.

TABLE 5. PERCENT MORTALITY IN *O. INSIDIOSUS* FEMALES MEASURED BY THREE METHODS IN 2001.

Insecticide	Rate kg ai/ha	Field[1]	Greenhouse[1]	Petri dish[1]
Untreated		16.3 gA	8.8 eA	15.0 dA
Spinosad	0.089	12.5 gB	11.3 eB	67.5 bcA
Spinosad	0.178	11.3 gB	12.5 eB	92.5 abA
Indoxacarb	0.07	43.8 efB	53.8 bB	82.5 abA
Indoxacarb	0.11	18.8 fgB	26.3 cdeB	88.8 abA
Imidacloprid	0.024	53.8 eB	38.8 bcdB	92.5 abA
Imidacloprid	0.047	58.8 cdeB	48.8 bcB	95.0 aA
Methoxyfenozide	0.25	20.0 fgAB	31.3 b-eA	5.0 dB
Methoxyfenozide	0.75	17.5 gA	17.5 deA	15.0 dA
Tebufenozide	0.125	20.0 fgA	15.0 deA	7.5 dA
Tebufenozide	0.25	22.5 fgA	20.0 deA	12.5 dA
Emamectin benzoate	0.0045	61.3 cdeB	82.5 aA	88.8 abA
Emamectin benzoate	0.009	82.5 abcA	91.3 aA	81.3 abcA
Abamectin	0.009	70.0 bcdA	85.0 aA	56.3 cA
Abamectin	0.018	85.0 abA	91.3 aA	75.0 abcA
Fipronil	0.038	83.8 abcA	90.0 aA	91.3 abA
Fipronil	0.05	78.8 a-dA	86.3 aA	77.5 abcA
λ-cyhalothrin	0.012	95.0 ab	97.5 aA	97.5 aA
λ-cyhalothrin	0.025	100.0 aA	98.8 aA	100.0 aA

Means within a column followed by the same lower case letter and means within a row followed by the same upper case letter do not significantly differ ($P \leq 0.05$, LSD).

[1]A total of 80 individuals were used per treatment.

Petri dish bioassay. Possibly, *O. insidiosus* did not receive a toxic dose in every instance in the treated plant bioassays (field and greenhouse) by not feeding on the treated plant during the 24-h exposure time used in this study. Another explanation offered is that the plant surface somehow altered or bound the pesticide deposits making them less available for uptake by the test insects.

TABLE 6. PERCENT MORTALITY IN *O. INSIDIOSUS* NYMPHS MEASURED BY THREE METHODS IN 2001.

Insecticide	Rate kg ai/ha	Field[1]	Greenhouse[1]	Petri dish[1]
Untreated		13.8 cA	17.0 cdA	12.5 cA
Spinosad	0.09	16.3 cB	26.3 cdB	85.0 abA
Spinosad	0.199	15.0 cB	23.8 cdB	75.0 bA
Indoxacarb	0.078	26.3 cB	42.5 bcB	70.0 bA
Indoxacarb	0.123	27.5 cB	40.0 bcB	93.8 abA
Imidacloprid	0.027	61.3 bB	31.3 bcC	100.0 aA
Imidacloprid	0.053	77.5 abA	48.8 bB	97.5 abA
Methoxyfenozide	0.28	22.5 cA	31.3 bcA	17.5 cA
Methoxyfenozide	0.84	18.8 cA	13.8 dA	12.5 cA
Tebufenozide	0.14	17.5 cA	22.5 cdA	13.8 cA
Tebufenozide	0.28	17.5 cA	18.8 cdA	25.0 cA
Emamectin benzoate	0.005	85.0 aA	93.8 aA	96.3 abA
Emamectin benzoate	0.01	90.0 aA	95.0 aA	100.0 aA
Abamectin	0.01	86.3 aA	97.5 aA	78.8 abA
Abamectin	0.02	87.5 aA	90.0 aA	96.3 abA
Fipronil	0.042	87.5 aA	93.8 aA	98.8 aA
Fipronil	0.056	95.0 aA	90.0 aA	100.0 aA
λ-cyhalothrin	0.014	100.0 aA	100.0 aA	98.8 aA
λ-cyhalothrin	0.028	100.0 aA	100.0 aA	100.0 aA

Means within a column followed by the same lower case letter and means within a row followed by the same upper case letter do not significantly differ ($P \leq 0.05$, LSD).

[1]A total of 80 individuals were used per treatment.

Imidacloprid and indoxacarb are known to have good translaminar movement into the leaf and would therefore move the pesticide away from direct contact to the insect in the plant bioassays. Because glass is an inert substance, it is not likely that the pesticide deposits would be altered or somehow bound to the substrate, leaving them free for uptake by an insect. Also, the entire inside surface of the dish was treated, making the parafilm cover the only area in which the insects could avoid the pesticide. In this study, test insects were observed on the inside of the parafilm cover only occasionally. In both the field and greenhouse bioassays, the clip cages offered a greater surface area on which the insects could avoid the pesticide. Even if the insect was not attempting to avoid the pesticide deposits, the chances of picking up a lethal dose would have been greater in the Petri dish. The most interesting results from this study were with spinosad. In both the field and greenhouse bioassays, mortality was not significantly different from that found in the untreated control, indicating that this pesticide is not toxic to *O. insidiosus*. However, mortality was very high in the Petri dish bioassay with this pesticide (100% in some instances). This leads one to think that the plant surface somehow makes this compound unavailable to this insect. Although spinosad is reported to have some translaminar movement into the leaf (Bret et al. 1997), this does not adequately explain the low toxicity in the plant bioassays.

Obviously, experimental design can have a pronounced effect on the outcome of a study and may offer some explanation on the disparity of results sometimes observed in the literature. In this study, particularly with spinosad, one would come to the conclusion that this pesticide would not be a good fit in a cotton IPM program with *O. insidiosus* when looking at the Petri dish bioassay alone. However, no effects were observed in the caged field and greenhouse studies, leading one to the opposite conclusion. It is apparent that merely evaluating mortality of pesticides on beneficial arthropods by only one method does not give an accurate depiction on how those pesticides would fit into IPM programs. This study concurs with Banken and Stark (1998) and Croft (1990) in that multiple testing methods should be used in evaluating pesticide effects on beneficial arthropods. However, this may not always be feasible. Often, lab studies utilizing an artificial substrate, are the quickest and least expensive means of obtaining data. However, particularly when working with omnivorous predators such as *O. insidiosus*, utilizing field studies or potted plants grown in the greenhouse would be the preferred method for bioassays. Bioassays utilizing artificial substrates, while providing important information, should not be the sole means of evaluating the effects of pesticides on beneficial arthropods.

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THE GENUS *RHOPALOSYRPHUS* (DIPTERA: SYRPHIDAE)

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ABSTRACT

The flower fly genus *Rhopalosyrphus* Giglio-Tos (Diptera: Syrphidae) is revised. The genus is redescribed; a key to species is presented; the phylogenetic relationships of the genus and species are hypothesized; the included species are described; with new species, *R. ramulorum* Weems & Deyrup, described from Florida (type) and Mexico; *R. australis* Thompson from Brazil and Paraguay (type); and the critical characters are illustrated.

Key Words: taxonomy, identification key, neotropics, nearctic

RESUMEN

El género de la mosca de la flor del género, *Rhopalosyrphus,* (DIPTERA: Syrphidae) es revisada y es redescrito; se presenta una clave para las especies; la relación filogenética del género y las especies es formulada; las especies incluidas son descritas; con las nuevas especies, *R. ramulorum* Weems & Deyrup, descrita de Florida (tipo) y México; *R. australis* Thompson de Brasil y Paraguay (tipo); y los caracteres críticos son ilustrados.

Translation provided by author.

Rhopalosyrphus Giglio-Tos is a small group of microdontine flower flies restricted to the New World subtropics and tropics, ranging from southern United States to northern Argentina. These flies are rarely collected, only some two dozen specimens are known. The adults mimic eumenine vespids, such as *Zethus*, which nest in twigs (Bohart & Stange 1965). The immature stages are known for only one species. These were found in ant nests (*Pseudomyrmex*) in twigs and grass culms, where the larvae probably prey on ant brood. The genus contains only three species. One wide ranging species, *R. guentherii*, is found from southwestern United States to northern Argentina. The others are more restricted in their ranges; *R. ramulorum*, presently known from a few specimens from Florida and Mexico, and *R. australis* from southeastern Brazil, Peru and Paraguay. The genus is here revised, with complete synonymies, descriptions, and distributional and biological data given for all taxa. Adult terminology follows Thompson (1999), larval terminology follows Hartley (1961) and Rotheray (1991).

GENUS *RHOPALOSYRPHUS* GIGLIO-TOS

Rhopalosyrphus Gilgio-Tos 1891: 3. Type species, *Holmbergia guentherii* Lynch Arribálzaga (subsequent monotypy, Giglio-Tos 1892a: 2). Williston 1892: 78 (catalog citation, descriptive note); Giglio-Tos 1892b: 34 [journal (1893: 130] (description); Aldrich 1905: 347 (catalog citation); Kertész 1910: 360 (catalog citation); Hull 1949: 312, figs. (description, figures of habitus, head, abdomen, hind leg); Capelle 1956 (review, key); Cole & Schlinger 1969: 307 (descriptive notes); Thompson et al. 1976: 60 (catalog citation); Vockeroth & Thompson 1987: 729 (key reference).

Holmbergia Lynch Arribálzaga 1891: 195. Type species, *guentherii* Lynch Arribálzaga (monotypy). Synonymy by Giglio-Tos (1892a).

Head: face convex, produced anteroventrally, pilose; gena small, linear, pilose; frontal prominence absent, antenna inserted above middle of head; frons short, about ¼ as long as face, as wide as face (♀) or slightly narrowed dorsally (♂), pilose; vertex broad, about 3 times as long as frons, as wide as frons, not swollen, pilose and punctuate; ocellar triangle small, equilateral, well separated from eye margins; occiput broad on dorsal ⅓; eye bare, dichoptic in male. Antenna elongate, longer than face; scape and basoflagellomere elongate, at least 4 times as long as pedicel; scape about 6 times as long as broad; arista bare, inserted basolaterally on mesal surface, about as long as scape.

Thorax: longer than broad; postpronotum pilose; meso-anepisternum with anterior portion not differentiated, uniformly pilose; meso-katepisternum completely pilose; meso-anepimeron with posterior portion bare; meropleuron with barrette

pilose; metasternum developed, pilose (although reduced in some species); scutum punctuate, with appressed pile; metatibia expanded apically; scutellum with or without small apical calcar, without distinct ventral pile fringe. Wing: brown on anterior 1/3, extensively microtrichious; marginal cell broadly open; stigmatic crossvein present; vein M1 with apical portion straight, joining vein R4+5 perpendicularly; vein M2 present or absent; vein R4+5 with spur.

Abdomen: petiolate; 1st segment short; 2nd segment as broad as thorax basally, but constricted, cylindrical apically; 3rd segment cylindrical; 4th and 5th segments forming a compact club; aedeagus bifid.

Puparium: elongate with broader ventral than dorsal surface; marginal band of variously-sized setae; dorsal surface flat; ventral surface convex; marginal band notched anteriorly; prothorax and mesothorax hidden beneath metathorax; mandible with serrate ventral margin.

Rhopalosyrphus belongs to the subfamily Microdontinae and is the sister group of *Ceriomicrodon* Hull, together these taxa are the sister group of *Microdon* Meigen, *sensu lato*. *Rhopalosyrphus* is defined (synapomorphy) by its 1) abdominal structure and 2) pilose meropleuron. Other diagnostic characters are 3) antenna elongate, longer than face, usually about twice as long; 4) scape and basoflagellomere elongate; 5) face produced ventrally; 6) occiput greatly developed on dorsal 1/3; 7) metasternum developed, not reduced; and 8) metatibia flared apically. The relationship to *Microdon*, *sensu lato* is unresolved: *Rhopalosyrphus* shares with the *Microdon* clade the bifid aedeagus and appears closely related to *Ceriomicrodon*. *Ceriomicrodon* shares characters 3, 4, 5, 6, 7, 8 and its abdominal shape could be considered derived from that of *Rhopalosyrphus*. The two differ only by the presence of pile on the meropleuron.

Microdon aurcinctus, described by Sack (1921: 138) in *Rhopalosyrphus*, belongs to the *Pseudomicrodon* group of *Microdon*. The species of this group differ from *Rhopalosyrphus* in the characters listed above and in having the vertex swollen and shiny.

Based on puparial characters, the immature stages of *Rhopalosyrphus* closely resemble *Microdon*. They both have the anterior end consisting of the metathorax, with the prothorax and mesothorax hidden beneath it, a marginal band of setae which surrounds the puparium except for a notch at the anterior end, sharply-pointed antennomaxillary organs and mandibles with a serrated ventral margin.

The very distinctive shape of the puparium of *Rhopalosyrphus* separates it from that of *Microdon*: it has a curved ventral surface that is broader than the narrow, flat dorsal surface. Also, the whole structure is elongate rather than oval in outline. The reverse appears in *Microdon*, with the dorsal surface being broader and curved and the ventral surface narrower and flat. These differences in shape suit the larva to life in hollow twigs and grass culms in which its prey, larvae and pupae of the ant, *Pseudomyrmex*, live.

Key to Species of *Rhopalosyrphus*

1. 3rd tergum short, about 1/3 as long as 2nd; 2nd tergum elongate (Fig. 9); eye with an area of enlarged ommatidia medially and posterior to antenna ..... *australis*

— 3rd tergum elongate, as long as 2nd; 2nd tergum not greatly elongate posteriorly (Fig. 10); eye without enlarged ommatidia 2

2. Alula completely microtrichose; cell R extensively microtrichose, bare only on basoposterior 1/4 or less; metasternum appearing bare, with pile greatly reduced; face and anepisternum partially black pilose ..... *ramulorum*

— Alula bare basomedially; cell R completely bare behind spurious vein; metasternum with long, distinct pile, not reduced; face and anepisternum entirely white pilose *guentherii*

Rhopalosyrphus guentherii Lynch Arribálzaga
Figs. 10-13

Holmbergia güntherii Lynch Arribálzaga 1891: 198, Fig. 3 (habitus) Argentina, Buenos Aires (T ♀ MACN lost?). Giglio-Tos 1892a: 2 (notes), 1893: 131 [sep. 35], pl. 1, Figs. 10, 10a-b (description, figures of abdomen, wing); Aldrich 1905: 347 (catalog citation); Kertész 1910: 360 (catalog citation); Fluke 1957: 36 (catalog citation); Capelle 1956: 172, Fig. 2 (description, synonymy, key reference, figure of head); Thompson et al. 1976: 60 (catalog citation).

Rhopalosyrphus carolae Capelle 1956: 174 A* ♂♀ Arizona, Huachuca Mts., Sunnyside Canyon (HT ♀ UKaL). Byers et al. 1962: 168 (HT UKaL); Wirth et al. 1965: 599 (catalog citation); Cole & Schlinger 1969: 307 (descr. note, distr. western N.A.); Thompson et al. 1976: 60. **NEW SYNONYM**

Wing length: 8.8 mm (♂)-10.5 mm (♀). Head: brownish black, yellowish white pilose; occiput grayish white pollinose on ventral 2/3, shiny dorsally; eye with ommatidia of more or less equal size; antenna brownish black except orange basal 1/3 of scape, about twice as long as face; antennal ratio 5: 1: 8.

Thorax: brownish black; pleuron silvery-white pilose; scutum brown pilose medially, silvery white pilose anteriorly, along transverse suture, laterally and posteriorly; scutellum silvery white pilose; calypter white, with brown margin and fringe; halter orange; wing brown anteriorly, hyaline posteriorly, microtrichose except bare cell R & BM, anterobasal 1/2 of cell CuP and basomedially on alula; legs reddish brown except yellow basal 1/2 of metafemur; pro- and mesofemora black pilose anteriorly, yellow pilose posteriorly; pro- and mesotibia yellow pilose; tarsi black pilose; metafemur black pilose with a few yellow pili intermixed; metatibia yellow pilose basally, black pilose apically.

Abdomen: brownish black except yellow basal 1/3 of 3rd tergum and reddish apically on 2nd tergum; 1st tergum white pilose; 2nd tergum constricted on apical 1/3, yellowish white pilose; 3rd tergum about twice as broad apically as basally, yellow pilose; 4th tergum brown pilose basomedial 2/3, yellow-white pilose apically, about as long as 2nd tergum; 5th tergum yellowish-white pilose.

Distribution: Texas (Cameron, Harris, Hidalgo, Kenedy and Kleberg counties); Arizona; Mexico (Chiapas, Colima, Michoacan, Morelos); Guatemala, Costa Rica, Peru, Brazil, Paraguay, Argentina (Lynch Arribálzaga).

Material examined: PARAGUAY: Colonia Nueva Italia, X-XI-1940, Pedro Willim (1 ♀ AMNH). BRAZIL: Amazonas: Parana do Xiboreninho, 03°15.S 60°00.W, mixed water, Canopy fogging project, TRS #60 Tray 392, 7-VIII-1979, Erwin, Adis & Montgomery (1 ♂ USNM ENT 00032864 USNM). PERU: Lambayeque, l km S Lambayeque, 24, 26-27-VII-1975, C. Parker & L. Stange (1 ♀ USNM ENT 0003863 FSCA). COSTA RICA: Alajuela, Cerro La Lana, San Ramón, 1200 m, LN 221750_481050, 17-I-1997, Betty Thompson, lot# 45327 (1 ♀ INBIOCRI002499628 INBIO); Guanacaste, Estación Exper. Enrique Jimenez Nuñex, 20 km SW Cañas, 5-17-XI-1991, Malaise Trap, A. S. Menke (1 ♀ USNM ENT 0003862 USNM); Puntarenas, Coto Brus. Sabalis, Estación El Progresso, Sector Fila Pizote, 1400 m, LS 317700_597800, 11-V-2001, M. Alfaro Libre, lot# 63200 (1 ♀ INB0003331118 INBIO). GUATEMALA: Alta Vera Paz, Trece Aguas, "Cacao," XI-1905, "GPColl" (1 ♂ USNM). MEXICO: [label just as "Mex."], (1 ♂ ANSP); Chiapas, Gutierrez, 20 miles S Tuxtla, 12-VIII-1963, F. D. Parker & L. A. Stange (1 ♂ USNM ENT 00032859 UCDavis); Colima, 6 km NE Tepames, 23-IX-Sept 1986, R. Miller & L. Stange (1 ♂ 1 ♀ USNM ENT 0003865-6 USNM, FSCA); Michoacan, Hidago, 12-VII-1963, F. D. Parker & L. A. Stange (1 ♀ USNM ENT 00032858 UCDavis); Morelos, 3 miles N Alpuyeka, 3400', 5-VI-1959, HE Evans (1 ♂ Cornell); …, Huajitlan, 27-IX-1957, R. & K. Dreisbach (1 ♀ USNM ENT 00032867 FSCA); Puebla, Chinantla, Sallé (1 ♀ UTOR). USA. ARIZONA: [Cochise/Santa Cruz Counties], Huachuca Mts, Sunnyside Canyon, 9-VII-1940, DE Hardy (allotype ♂, UKaL). TEXAS: Hidalgo Co: Pharr, 23-VI-1947 (1 ♀ USNM); La Joya, 19-III-1970, J, O'Grady (1 ♀ USNM), Rio Grande Park, 10 July 1981, A. Hook (1 ♀ USNM); … 12-VII-1981, A. Hook (1 ♀ USNM); McAllen, Valley Botanical Garden, 28-III-1975 (2 ♂ USNM ENT 0003868-9 FSCA), … 20-III-1976 (1 ♀ USNM ENT 0003870 FSCA), … 3-IV-1975 (1 ♀ USNM ENT 0003871 FSCA), … 5-IV-1975 (1 ♀ USNM ENT 003872 FSCA), … 2-IX-1975 (1 ♂ USNM ENT 0003873 FSCA); Relampago, 17-X-1986, FC Fee (1 ♂ 1 ♀ Fee) flying around flowering shrub, *Schinus* sp.; Santa Ana N.W.R., 17-X-1984 (1 ♀ Fee); Madero, 18-X-1995, FD Fee (1 ♂ Fee), 11-XI-1995, FD Fee (1 ♀ Fee) collected on flowers of composite shrub, *Gochnatia hypoleuca* DC. Kenedy Co.: 27°10.N 97°40.W, 8-X-1975, J.E. Gillaspy (1 ♂ USNM). Cameron Co.: Brownsville, Los Palamos Mgt Area, 17-X-1976, FD Fee (1 ♀ USNM, 2 ♂ Fee); Brownsville, VI, ("Cata 1439" Brooklyn Mus Coll 1929 (1 ♀ USNM); Brownsville, 23-X-1976, FD Fee (1 ♂ 2 ♀ Fee); Sabal Palm Grove Sanctuary, 9-X-1986, FD Fee (1 ♀ Fee), … 20-X-1986, FD Fee (4 ♂ 1 ♀ Fee), all individuals fly about, attracted to, or feeding on exudate from glands at base of leaves of sapling trees of *Ehretia anagua* (Teran & Berl.) I. M. Johson; … 27-III-1988 (1 ♂ Fee), 5-IV-1988 (1 ♂ Fee) flying about or attracted to flowers of *Zanthoxylum fagara* (L.) Sarg.; … 21-XI-1995, FD Fee (1 ♂ Fee). Harris Co.: Houston, 45 W Virginia Str., 28-VIII-1969, at black light trap, TJ Henry (1 ♂ USNM). Kleberg Co.: Kingsville, South Pasture, 26-IX-1976, at Baccharis, JE Gillaspy (1 ♂ USNM)

The traditional nomenclature and taxonomy of *Rhopalosyrphus* are maintained. Giglio-Tos established that there was a single widespread species, ranging from Mexico to Argentina, and that the appropriate name for that taxon was *Rhopalosyrphus guentherii*. The specimens studied support the single widespread taxon concept of Giglio-Tos. That the appropriate name for the taxon is *quentherii* is not as certain, as the holotype of *quentherii* has not been found and nothing mentioned in the original description will unequivocally allow the assignment of that name to either the widespread taxon or *australis*. The types of *Rhopalosyrphus carolae* Capelle were examined and are representative of the widespread taxon (**new synonym**).

Rhopalosyrphus australis Thompson, **new species**
Fig. 9

Wing length: 10 mm (♂) - 11 mm (♀). Head: Face reddish to brownish black (except holotype broadly yellowish dorsolateral), always black medially, white pilose except for a few black pili ventrally; gena small, linear, white pilose; frons and vertex black, white pilose; occiput reddish brown, white pollinose and pilose on ventral 1/3, shiny

dorsally; eye with a medial fasciate area of enlarged ommatidia; antenna about 1.5 times as long as face, antennal ratio 5:1:10.

Thorax: brownish black; pleuron white pilose; mesonotum punctate, very short appressed pilose, white pilose in males, more extensively black pilose medially in females; metasternum with short, appressed pile; calypter white, with brown margin and fringe; halter orange with brown head; wing brown anteriorly, hyaline posteriorly, microtrichose except bare cell R & BM, anterobasal 1/2 of cell CuP and basomedially on alula; legs reddish brown except yellow basal 2/3 of metatibia, pale pilose except black pilose dorsomedially on pro- and mesotibiae and tarsi, with dense black spinose pile on ventrolateral 2/3 of metafemur, with ventromedial appressed black spinose pile on basal 2/3 of metatibia.

Abdomen: black except yellow 3rd tergum and reddish apically on 2nd tergum, mainly short appressed white pilose, except black pilose medially on 4th tergum; 1st short, as long as 3rd; 2nd tergum half as long as entire abdomen, constricted and cylindrical on apical 2/3, as wide as thorax basally; 3rd tergum short, as long as 1st; 4th tergum oval, as long as 2nd, forming with 3rd a distinct club in ♂; 5th tergum elongate, about 1/2 as long as 2nd, forming with 3rd and 4th a distinct club in ♀.

Distribution: Peru, southern Brazil and Paraguay.

Holotype ♀: PARAGUAY, Villarica, I-1939, F. Schade, deposited in the American Museum of Natural History, New York. Paratypes: BRAZIL: Ceara, Russa, s., II-1940, D. C. Alves (1 ♀ USNM); Ceara, Limoeiro, X-1938, R. C. Shannon (1 ♂ USNM); Ceara, Luixeramobug, XI-1940, D. C. Alves (1 ♂ MZUSP); Minas Gerais, Belo Horizonte, 800 m, Estacao Ecológica, UFMG Campus, clear trail 60 m in from road, near swamp, Malaise trap, S. D. Gaimari, 25-29-V-1993 (1 ♂ USNM ENT 00032860 USNM), … 15-18-VI-1993 (1 ♂ USNM ENT 00032860 USNM). PERU: Junin, Colonia Perene, Rio Perene, 18 miles NE La Merced, 3-I-1955, E. I. Schlinger & E. S. Ross (1 ♀ USNM ENT 00030699 CAS).

Rhopalosyrphus australis is readily distinguished from the other two species of *Rhopalosyrphus* by its distinctive abdominal shape. The epithet, *australis*, refers to the southern distribution of the species and is an adjective.

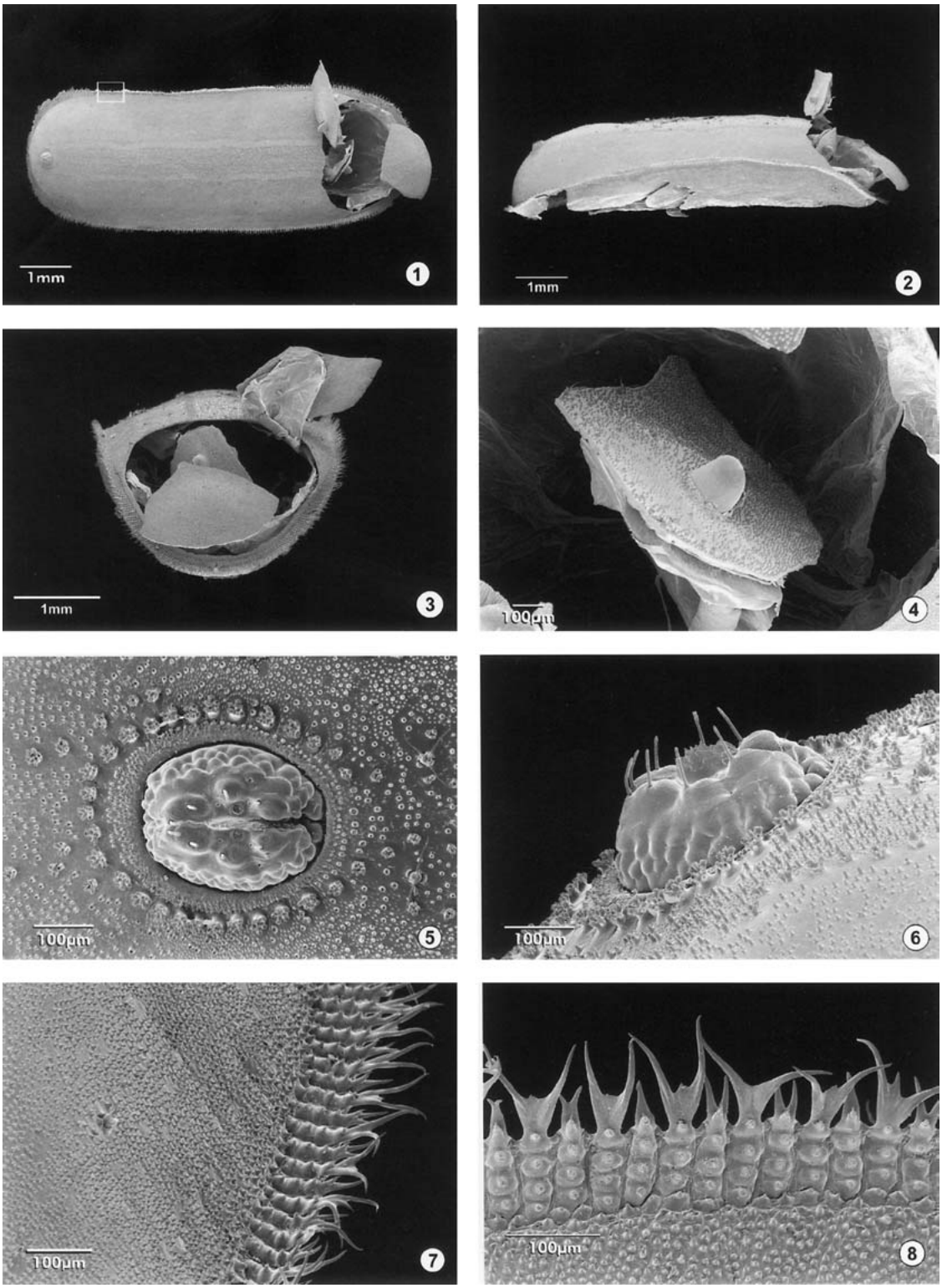
Rhopalosyrphus ramulorum Weems & Deyrup,
new species
Figs. 1-8, 14

Wing length: 6 mm (♂)-7 mm (♀). Head: black; face silvery white pilose, with a few dark pili medially; frons sparsely white pilose, with a distinct bare fascia dorsally and separating off vertex; gena white pilose; vertex silvery white pilose, with a few dark pili medially; eye with ommatidia of more or less equal size; occiput white pilose on ventral 2/3, shiny dorsally; antenna brownish black except orange basal 1/3 of scape, about 1.5 times as long as face; antennal ratio 5: 1: 7 ♂ 4: 1: 5 ♀.

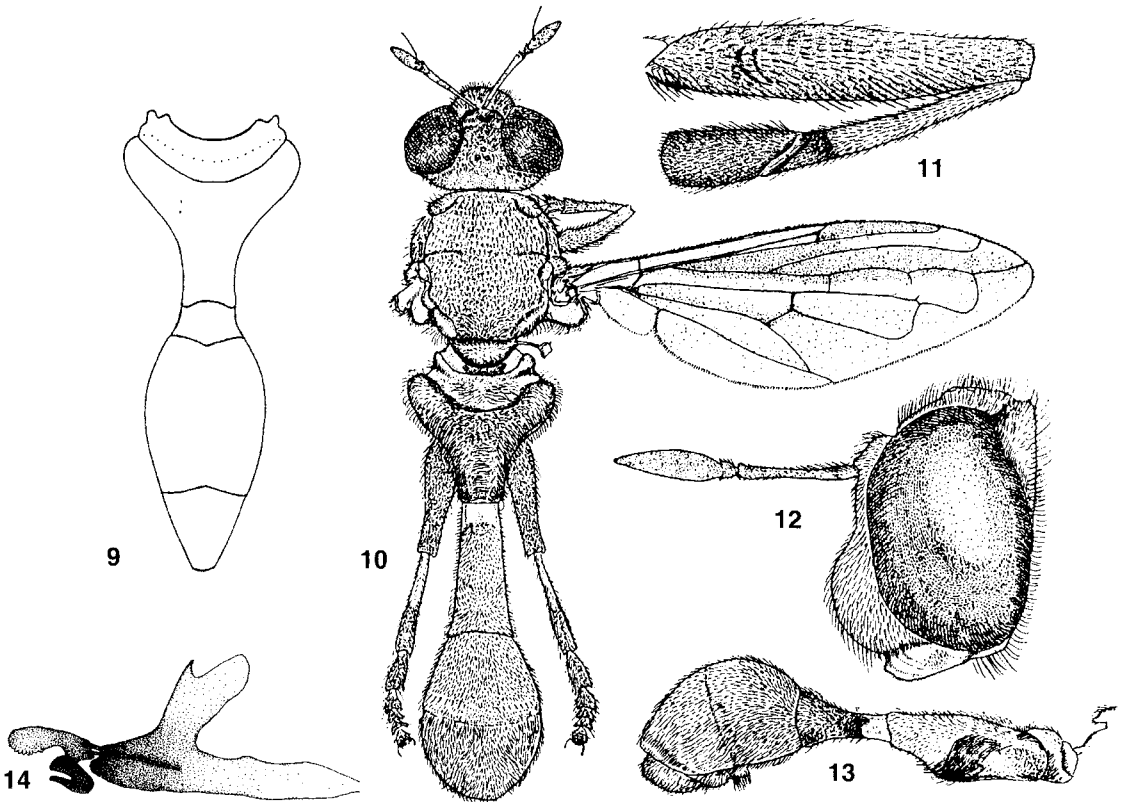
Thorax: black, silvery white appressed pilose on pleuron except with some dark pili on anepisternum; scutum dark appressed pilose except silvery white pilose anteriorly, along transverse suture and anterior to scutellum; calypter white, with brown margin and fringe; halter yellow; legs dark brownish black, except yellow basal 1/3 of pro- and mesotibia and basal 2/3 of metatibia, black pilose except pale pilose on pale areas. Wing: extensively dark fumose, except paler on posterior 1/3, microtrichose except bare basal 1/3 of cell R.

Abdomen: black except very narrowly yellow basolaterally on 3rd tergum; 1st tergum black pilose laterally, white pilose medially; 2nd tergum constricted on apical 1/3, black pilose on basal 2/3, white apically; 3rd tergum about twice as broad apically as basally, black pilose on basal 1/2, yellowish white pilose apically; 4th tergum about as long as 2nd tergum, black pilose on basal 1/2 and extending to posterior 1/3 laterally, yellowish white pilose on apicomedial 1/2 and apicolaterally; 5th tergum yellowish white pilose.

Puparium: 7.5 mm, width 2.5 mm; semi-circular in cross-section with a ventral surface about twice as broad as dorsal surface; elongate, nearly 3 times as long as broad; antennomaxillary organs (extracted from puparium) sharply pointed; prothorax and mesothorax retracted into metathorax so that structures associated with mouthparts not visible; anterior margin of puparium consisting of metathorax; cephalopharyngeal skeleton (extracted from puparium, Fig. 14) similar to *Microdon* (Garnett et al. 1990); mandible blade-like, with serrated ventral margin, with rounded tip; abdominal segments with 5 dorsal (about marginal band of setae) groups of sensilla, each group with 2-3 terminal setae, 4 ventral groups which lack terminal setae, with some sensilla group on two separate papillae; marginal band composed of 2 types of papillae alternating with each other: larger papillae with 3 terminal setae and smaller papillae with 2 terminal setae (Fig. 8); papillae comprising marginal band longer and packed close together on anterior and posterior ends of puparium; above these alternating bands of papillae 2 rows of short papillae, about as long as those bearing sensilla; marginal band interrupted only on anterior margin of metathorax; dorsal surface coated in small, dot-like papillae aggregated into vague reticulate pattern; ventral surface smooth, lacking setae and papillae; mid-dorsal region of abdominal segments 2-7 with 6 longitudinal rows of larger papillae, between outer 2 rows dot-like papillae densely ag-



Figs. 1-8. Puparium of *Rhopalosyrphus ramulorum*. 1-3, habitus, 1, dorsal, 2, lateral, 3, anterior. 4, anterior spiracular process, lateral. 5, 6, posterior respiratory process. 5, dorsal view and surrounding papillae, anterior end uppermost; 6, lateral view, anterior end to the left. 7-8. Papillae from marginal band.



Figs. 9-14. Features of *Rhopalosyrphus*. 9, abdomen, *australis*, dorsal; 10. Habitus *guentherii*, dorsal; 11, hind leg, *guentherii*, lateral; 12. head, *guentherii*, lateral; 13. abdomen, *guentherii*, lateral; 14. cephalopharyngeal skeleton, *ramulorum*, lateral. Figures 10-13 from Hull (1949).

gregated creating impression of vague pair of vittae running along dorsal surface (Fig. 1). Posterior respiratory process (Fig. 6): 0.3 mm long, 0.2 mm high, oval, nodulate with mid-dorsal projection, surrounded by papillae, with 4 pairs of interspiracular setae and 3 pairs of spiracular openings (Fig. 5).

Distribution: USA (Florida) south to Mexico (Chiapas).

Holotype ♂: USA: Florida, Highlands Co., Lake Placid, Archbold Biological Station, Trail 1 SSo, 22-V-1985, Malaise Trap, M. Deyrup, deposited in the National Museum of Natural History (USNM), Washington. Paratypes: USA. FLORIDA, same locality as holotype, 5-V-1986, reared from nest of *Pseudomyrmex simplex* in twig of *Carya floridana*, M. Deyrup (1 ♀ USNM); …, L. L. Lampert, Jr. & H. W. Weems, Jr., 8-IV-1978 (2 ♂ USNM ENT 0003891-2 FSCA); …, 11-IV-1978 (1 ♀ USNM ENT 0003893 FSCA); …, 18-III-1975, H. W. Weems, Jr. (1 ♂ USNM ENT 0003894 FSCA); …, 17-IX-1979, T. A. Webber & H. W. Weems, Jr. (1 ♀ USNM ENT 0003895 FSCA); F. E. Lohrer, H. W. Weems, Jr., 11-15-IV-1980 (1 ♀ USNM ENT 0003896 FSCA), …, 21-22-IV-1980 (1 ♂ USNM ENT 0003897 FSCA); …, 14-15-V-1980 (1 ♂ USNM ENT 0003898 FSCA); …, 18-20-V-1980 (1 ♂ USNM ENT 0003899 FSCA); …, Highlands Hammock State Park, 3-IV-1965, H. Weems, Jr. (1 ♀ USNM ENT 0003877 FSCA); …, 27-III-1966 (1 ♀ USNM ENT 0003878 FSCA); Collier County, SR94, 1.8 miles south of US 41, 25-II-1992, M. Deyrup & B. Ferster, reared from nest of *Pseudomyrmex ejectus* in culm of *Cladium jamaicense* (ABS); Liberty Co., Torreya State Park, 14-V-1964, H. Weems, Jr. (1 ♀ USNM ENT 0003875 FSCA); …, 30-IV-5-V-1973, C. R. Artaud & H. Weems, Jr., Malaise trap, (1 ♂, 4 ♀♀ USNM ENT 0003879-83 FSCA, USNM); Alachua Co., Gainesville, Beville Heights, L. A. Stange, Blacklight, 2-VII-1980 (2 ♀♀ USNM ENT 0003886-7 FSCA); …, 1-VII-1980 (1 ♀ USNM ENT 0003888 FSCA); …, 5-VII-1980 (1 ♂ USNM ENT 0003889 FSCA); …, 30-VII-1979 (1 ♀ USNM ENT 0003890 FSCA); Dade Co., Ross & Castello Hammock, 30-III-1963, C. F. Zeiger (1 ♂ USNM ENT 0003884 FSCA); …, Fuch's Hammock, near Homestead, 27-29-VII-1978, Terhune S. Dickel & H. Weems, Jr. (1 ♀ USNM ENT 0003885 FSCA); Chekika State Recreation Area, 10-XI-1982, FD Fee (1 ♂ Fee). MEXICO. Morelos, 3 miles N Alpuyeka, 3400', 5 June 1959, HE Evans, 14-V-

1959, Biol. Note 601 (1 ♂ USNM ENT 0003876 FSCA); Chiapas, 28 miles west Cintalpa, 9-IV-1962, F. D. Parker (1 ♂ USNM). Another broken ♂ specimen is in the Canadian National Collection and is labelled "Letitia, Colombia? (or Florida)." According to Vockeroth (pers. comm.) this specimen was found in a Malaise trap which had been used both in the Florida Keys and Colombia.

Rhopalosyrphus ramulorum is similar to *guentherii*, but is smaller, narrower, not as robust, and has much more extensive black pile on face, scutum and anepisternum. The metasternal pile is greatly reduced and closely appressed, so the metasternum appears bare at low magnifications.

Although many specimens of *R. ramulorum* have been collected, two of these are reared specimens and provide most of our insights into the natural history of the genus *Rhopalosyrphus*. When alive, the reared adults, like many other syrphids, bore a strong resemblance to stinging Hymenoptera. The wasp-like features of elongate antennae, narrow abdominal "petiole," pale bands and spots, and dark wings are enhanced by the wasp-like habit of holding the wings out from the body. The wings are also partially folded, so that they appear long and narrow. The general impression is of a very small individual of the twig-nesting eumenid genus *Zethus*.

One specimen was found in a nest of the ant *Pseudomyrmex simplex* in a small twig (hence the species epithet "*ramulorum*," the epithet to be treated as a noun in the genitive case) of *Carya floridana* in long unburned Florida scrub habitat. The adult emerged from its pupa the day after the twig was opened. A second specimen was in a nest of *P. ejectus* in a culm of *Cladium jamaicense*; this adult also emerged a day after the nest was opened. The nests of these two species of *Pseudomyrmex* are kept clean and free of debris and fungi, and it is probable that the fly larvae are not scavengers, but predators feeding on ant brood. This would fit well with the known larval habits of the closely related genus *Microdon*, (Duffield 1981; Garnett et al. 1985). This is apparently the only known example of a predatory inquiline attacking members of the large neotropical ant genus *Pseudomyrmex*, though there must be others, especially among the Eucharitidae. *Pseudomyrmex* species are less susceptible to inquilines than most ants because the nests are in plant cavities with access by only one or a few small, well-guarded holes, and the nests themselves are bare, with minimal edible detritus and no hiding places for inquilines.

The holes used by *Pseudomyrmex simplex* and *P. ejectus* are much too small to permit the adult fly to escape, and it seems probable that emergence from the puparium is delayed until the nest has been broken open. Since the term "strategy" has been used extensively in discussing *Microdon* (Duffield 1981), the problem of adult egress is a major flaw in the strategy of *R. ramulorum*. It may be, however, that there are ways an ovipositing female can increase the likelihood that her offspring will be freed. Small, dead, exposed twigs are more likely to get broken off than larger twigs in the interior of the tree crown. Culms on the edge of a tussock of sedge or grass are more likely to get broken off than culms in the interior. If there is some special site selection by the female, this may explain why only two pupae were found in a 10-year study of Florida ants, a study that involved opening hundreds of colonies of *Pseudomyrmex*.

This brings up the topic of apparent rarity of *Rhopalosyrphus* species, especially in Florida. At the Archbold Biological Station, two Townes traps running continuously for 3 years captured only one specimen. If the adults spend their time in the tops of trees or in extensive open marshes, this would explain why so few specimens appear in Malaise traps, which are usually set-up in understory flyways. If *R. ramulorum* is actually dependent on chance events to release the adults, actual populations would need to be quite high for the sexes to meet, even if there were a mechanism for adult aggregation, and even if some synchronous emergence were provided by wind storms. Whatever the actual abundance of *Rhopalosyrphus* species, the rarity of specimens in collections suggests that there could be additional undiscovered species, especially in the neotropics, where the fauna of arboreal ants is large.

Acknowledgments

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EFFECT OF SCREENING METHODS ON EXPRESSION OF ROMAINE LETTUCE RESISTANCE TO ADULT BANDED CUCUMBER BEETLE, *DIABROTICA BALTEATA* (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

Resistance in lettuce, *Lactuca sativa* L., to feeding by adult banded cucumber beetle, *Diabrotica balteata* (LeConte), was evaluated using three screening methods: leaf disks, excised leaves and intact leaves attached to plants. Dual-choice and no-choice bioassays were used to evaluate each method based on leaf area consumption. Methods of testing had a significant effect on the level of feeding damage by *D. balteata* on two lettuce cultivars, Tall Guzmaine and Valmaine. Valmaine expressed a significant degree of resistance to *D. balteata* damage when intact leaf and excised leaf methods were used in dual-choice bioassays between Tall Guzmaine and Valmaine, but the latter failed to show resistant characteristics in no-choice tests when excised leaves were used. Furthermore, there was no significant difference in *D. balteata* feeding between Tall Guzmaine and Valmaine in the leaf disk tests. Therefore, whole plants are the best method to evaluate lettuce cultivars for resistance to *D. balteata*. Reduction or cessation of resistance characters in excised Valmaine whole leaves and disks are discussed with references to potential changes in concentration of feeding stimulants and deterrents and changes in latex pressure.

Key Words: *Lactuca sativa*, leaf disk, excised leaves, intact leaves

RESUMEN

La resistencia en la lechuga, *Lactuca sativa* L., hacia la alimentación de adultos del escarabajo rayado del pepino, *Diabrotica balteata* (LeConte), fué evaluada usando tres métodos de seleccionar: hojas cortadas en forma de disco, hojas cortadas, y hojas intactas pegadas a la planta. Se utilizaron un bioensayo de una prueba de doble opción y de una prueba sin opción para evaluar cada método basado en el consumo del área de la hoja. Los métodos de prueba tienen un efecto significativo sobre el nivel de daño causado por la alimentación de *D. balteata* en dos variedades cultivadas de lechuga, la "Tall Guzmaine" y la "Valmaine". La variedad Valmaine expresó un grado de resistencia significativo al daño de *D. balteata* cuando fueron usados los métodos de las hojas intactas y de las hojas cortadas en los bioensayos de doble opción entre las variedades Tall Guzmaine y Valmaine, pero la última no mostró características de resistencia en pruebas de una sola opción cuando se usaron hojas cortadas. Además, no habia una diferencia significativa entre la Tall Guzmaine y la Valmaine en cuanto de la alimentación de *D. balteata* en pruebas de hojas cortadas en forma de discos. Por lo tanto, las plantas enteras son el mejor método para evaluar las variedad de lechuga para su resistencia al *D. balteata*. Se discuten la reducción o el paro de las características resistentes en hojas cortadas de la Valmaine en hojas enteras y en de hojas cortadas en forma de discos con referencia a los cambios potenciales en la concentración de estimulantes y disuacivos de alimentación y cambios en la presión de latex.

Host plant resistance is recognized as an effective component of IPM (Panda & Khush 1995), because it has low impact on non-target organisms and the environment and is usually compatible with other control tactics. Reliable and efficient screening techniques are essential for accurately evaluating resistance levels. Excised leaves or leaf disks are often used for evaluating plants for resistance to leaf feeding insects. For example, Sams et al. (1975) found that the use of excised

leaflets was an efficient method for evaluating resistance to green peach aphid (*Myzus persicae* (Sulzer)) in tuber-bearing *Solanum* germplasm. Excised leaves were found to be quite reliable for screening bean cultivars for Mexican bean beetle (*Epilachna varivestis* Mulsant) feeding preference as long as large mature leaves were used (Raina et al. 1980). The leaf disk and whole leaf techniques worked equally well for screening resistance to two-spotted spider mites (*Tetranychus urticae* Koch) on muskmelon leaves (*Cucumis melo* L.) (East et al. 1992). However, some screening methods may have a significant impact on the test outcome by altering natural resistance mechanisms. Risch (1985) found that screening methods affected the expression of resistance and the order of feeding preference among corn (*Zea mays* L.), bean (*Phaseolus vulgaris* L.) and squash (*Cucurbita pepo* L.) to specialist and generalist chrysomelid beetles.

In this study, we evaluated the effect of three screening methods (leaf disks, excised leaves and intact leaves attached to plants) on the expression of resistance in romaine lettuce cultivars to feeding by adult banded cucumber beetles (*Diabrotica balteata*). Leaf area consumed by beetles was evaluated in dual-choice and no-choice bioassays to compare results for both leaf disks and detached leaves against intact leaves.

Materials and Methods

Plants and Insects

A previous study showed that Valmaine (Val) was the most resistant cultivar and Tall Guzmaine was the most susceptible one to *D. balteata* feeding among four lettuce cultivars (Huang et al. 2002). Therefore, Valmaine and Tall Guzmaine were used for this experiment. Seeds of each cultivar were kept overnight in the laboratory in separate petri dishes lined with wet filter paper for better germination. Germinated seeds were planted in a transplant tray filled with a commercial soil mix (MetroMix 220, Grace Sierra, Milpitas, CA) and grown for 2 wk in a greenhouse with natural light. Seedlings were transplanted to 10-cm diameter plastic pots filled with MetroMix 220. Each plant was watered daily and fertilized weekly with 10 ml of a 10 g/L solution of a soluble fertilizer (Peters 20-20-20, N-P-K, W. R. Grace, Fogelsville, PA) from transplantation until the end of the experiment. Fully expanded leaves from the seventh position (counting from the first true leaf) were used in all the assays and selected plants had seven to eight fully expanded leaves.

Adult *D. balteata* for feeding bioassays were obtained from a laboratory colony originally collected from the field in Belle Glade, Florida in June 1996. Adults were fed lima bean leaves and sweetpotato tubers and larvae were reared on corn seedling roots as previously described (Huang et al. 2002). Only unfed adults which had emerged within 48 h were used for the assays. All tests were conducted in a rearing room at 25 ± 1°C, 14:10 (L:D) h photoperiod.

Intact Leaves (Experiment 1)

Dual-choice tests were first conducted using intact leaves of Tall Guzmaine and Valmaine plant pairs. Feeding arenas made from plastic petri dishes (8.9 cm diameter) were attached using hair clips to the upper leaf surface of a pair of leaves from each cultivar. Two round holes (2.9 cm diameter) that were 65 mm apart provided access to the leaves, and a 5.8 cm diameter hole that was covered with gauze material at the top of the dish provided ventilation. One pair (female plus male) of beetles was placed in each feeding arena and allowed to feed for 48 h. Each test was replicated 11 times. The extent of feeding was evaluated by scanning the leaf material (JADE 2, Linotype-Hell, Taiwan) and importing the resulting images into an imaging program (ImagePC beta version 1, Scion Corporation, Frederick, Maryland) where leaf area consumed (mm^2) was determined. The difference in leaf area consumption between cultivars was analyzed by paired *t*-test using Proc MEANS (SAS Institute 1999).

Excised Versus Intact Leaves (Experiment 2)

Resistance to beetle feeding was next compared between excised and intact leaves. The petioles of individual leaves excised at their base were immediately immersed in separate beakers filled with tap water and maintained therein for the duration of testing. Dual-choice feeding arenas as described above were used to expose single pairs of adults (female plus male) for 48 h to pairs of either excised or intact Tall Guzmaine and Valmaine leaves. Each test was replicated 17 times in each bioassay. The difference in leaf area consumption between cultivars was estimated and analyzed as described above.

Since a significant difference was found in leaf area consumption between the two cultivars in dual-choice tests, the resistance level of excised and intact leaves was further evaluated using no-choice bioassays. Excised and intact leaves were chosen and prepared as above. One pair of female and male beetles was confined on individual excised or intact leaves of a single cultivar using a modified feeding area with only a single 4 cm diameter hole through which beetles accessed the upper leaf surface. The adults were allowed to feed for 48 h. This study was arranged as a randomized complete block design with excised and intact leaves from each cultivar in each block. Each block was replicated 18 times. Leaf area consumed was estimated as described above and

analyzed by Proc GLM (SAS Institute 1999). Means with significant ANOVA were separated using Tukey's HSD test with a significance level of $\alpha = 0.05$ (SAS Institute 1999).

Leaf Disks

Leaf disks for the bioassays were harvested from freshly excised Valmaine or Tall Guzmaine leaves. Two 380 mm^2 disks were punched out from non-midrib areas of each leaf using a No. 15 corkborer. The bioassay consisted of four leaf disks (two from Tall Guzmaine and two from Valmaine) placed an equal distance apart on two layers of moistened paper towel inside a 8.9 cm diameter plastic petri dish. A female and male beetle was placed in each petri dish and allowed to feed for 48 h. Bioassays were replicated 15 times. Leaf area consumed was estimated as described above, but the remaining leaf area was subtracted from the mean disk area of 10 disks not offered to beetles for two days in order to account for shrinkage during the assay. The difference in leaf area consumption between cultivars was analyzed by paired *t*-test using Proc MEANS (SAS Institute 1999).

Results

Valmaine was strongly resistant to beetle feeding compared with susceptible Tall Guzmaine when intact or excised leaves were presented in dual-choice tests (experiments 1 and 2, Table 1). However, beetles ate significantly more from excised leaves of both cultivars compared to intact leaves (experiment 2, $t = 3.88$, $df = 32$, $p < 0.0001$). Beetles on intact leaves consumed 12-fold less from Valmaine compared to Tall Guzmaine, while on excised leaves consumption was only 4-fold less. When leaf disks were used as test materials, there was no significant ($p \geq 0.05$) difference in feeding between Tall Guzmaine and Valmaine. Adults ate up to 23 times as much Valmaine on leaf disks as on intact leaves (experiment 1). Mean leaf area consumed on leaf disks and intact leaves of Tall Guzmaine was similar, and both were over 318 mm^2.

A significant difference among treatments in leaf area consumed per pair of adults in no-choice tests was also found ($F = 21.78$; $df = 3, 51$; $P = 0.0001$) (Fig. 1). Beetle pairs consumed significantly less (81%) Valmaine than Tall Guzmaine on intact leaves, but consumed similar leaf areas from excised leaves. Feeding was significantly increased on excised leaves compared to intact leaves, irrespective of cultivar. Intact Valmaine leaves were the least damaged by adult *D. balteata* with mean leaf area consumption of 66.3 mm^2, which was only 12% of that on excised Valmaine leaves.

Discussion

Although excised leaves or leaf disks are often used for evaluating plants for resistance to leaf feeding insects, biochemical and physiological changes in such plant tissue may affect the feeding of the test insects (Raina et al. 1980; Risch 1985; van Emden & Bashford 1976). In our case, Valmaine expressed a higher degree of resistance to adult feeding on intact leaves than on excised leaves in choice tests. Moreover, Valmaine failed to show significant resistance to *D. balteata* feeding in either the leaf disk choice test (Table 1) or the excised leaf no-choice test (Fig. 1). Therefore, methods of testing for resistance had a significant effect on relative leaf consumption of Tall Guzmaine and Valmaine by adult *D. balteata*. The intact leaf method was the most suitable and reliable of the tested methods used to evaluate lettuce cultivars for resistance to *D. balteata*.

Risch (1985) also reported that testing method (i.e., whole plants, excised leaves, and leaf disks) had a significant effect on preferences of chrysomelid beetles, including *D. balteata*, when corn, bean and squash were tested. In his tests, differences in resistance ratios between leaf disk and whole plant tests were much greater than those between excised leaves and whole plant tests.

TABLE 1. MEAN ±SEM LETTUCE LEAF AREA CONSUMED PER PAIR (FEMALE PLUS MALE) OF ADULT *D. BALTEATA* IN 48 H WHEN PRESENTED A CHOICE BETWEEN TWO CULTIVARS USING DIFFERENT SCREENING METHODS

Methods	Cultivar[a]	N	Leaf area (mm^2)	Pr > |t|
Intact leaf (expt 1)	TG	11	318.1 ± 29.3	0.0001
	Val	11	14.4 ± 2.1	
Intact leaf (expt 2)	TG	17	350.5 ± 28.7	0.0001
	Val	17	28.8 ± 4.8	
Excised leaf	TG	17	532.2 ± 57.8	0.0004
	Val	17	125.9 ± 53.8	
Leaf disk	TG	15	382.8 ± 36.3	0.0941
	Val	15	334.6 ± 37.4	

[a]TG = Tall Guzmaine, Val = Valmaine.

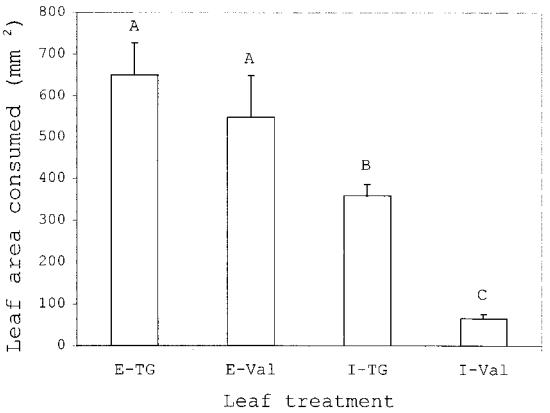

Fig. 1. Mean leaf area consumed per pair of adult *D. balteata* within 48 h during no-choice test using excised (E) and intact (I) leaves from susceptible Tall Guzmaine (E-TG, I-TG) and resistant Valmaine (E-Val, I-Val). Bars topped with the same letter are not significantly different by Tukey's HSD test at the 0.05 level. Vertical lines indicate + 1 SEM.

Furthermore, the feeding preferences of the two specialist species, *Acalymona thiemei* (Baly) and *Ceratoma ruficornis* (Olivier) were less affected by test method than were the more generalist species, *D. balteata* and *D. adelpha* (Harold). In another example of different results between intact and excised leaf tissue, lettuce cultivars normally resistant to the lettuce aphid, *Nasonovia ribisnigri* (Mosley), lost their resistance when leaf fragments were given in a leaf disk test (Schoonhoven et al. 1998).

Leaf disk size is another variable that could affect the outcome of insect feeding preferences. The ratio of cut edge to overall leaf disk surface area can influence the chance of encountering internal attractants and stimulants (Jones & Coleman 1988).

The fact that leaves of both Valmaine and Tall Guzmaine were consumed much more when excised from the plants suggests a change in the chemical profile inside leaves or a reduced capacity to deliver deterrents effectively after cutting. Latex in some laticiferous plants has been reported as a natural defense system against certain herbivores. In many laticiferous plants, including *L. sativa*, latex is stored under pressure within laticifers, which results in rapid release of latex upon cutting (Fahn 1979; Data et al. 1996; Dussourd 1995). The secretions often contain secondary metabolites known to be toxic or deterrent to animals (Farrell et al. 1991). Data et al. (1996) found that young vine material of sweet potato produced more latex and had fewer sweetpotato weevils, *Cylas formicarius* (F.), than older and more mature portions of the vine. Several insects have been observed immobilized in exudates, such as caterpillars (Dussourd 1993), ants (Dillon et al. 1983), aphids and whiteflies (Dussourd 1995). Many different organic compounds have been identified in latex of *Lactuca* sp., including organic acids, phenolics and a triterpene alcohol (Crosby 1963; Gonzalez 1977; Cole 1984). Like many plant secondary compounds these organic compounds may act as deterrents or toxins to potential herbivores. Both Valmaine and Tall Guzmaine produce latex upon cutting, but latex flows from Valmaine longer after cutting than from Tall Guzmaine (Huang et al. 2003). Beetles may have eaten more on excised than on intact leaves because latex flow from injured tissue on excised leaves placed in water may be decreased and diluted compared to intact leaves. No latex emission was observed from leaf disks which may be the major reason why no feeding difference was found between Valmaine and Tall Guzmaine. However, Tall Guzmaine was preferred over Valmaine by beetles when excised leaves were used in choice tests and when intact leaves were used in both choice and no-choice tests. Therefore, the observed feeding preferences may be due to differences in the composition or concentration of secondary compounds within the latex between Tall Guzmaine and Valmaine.

Acknowledgments

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SURVEY OF PEST MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE) ACTIVITY ON PASTURE IN SOUTH-CENTRAL FLORIDA

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Abstract

Histories of pest mole cricket activity (*Scapteriscus* spp. Scudder) (Orthoptera: Gryllotalpidae) on bahiagrass (*Paspalum notatum* Fluegge) pastures were needed to provide baseline data for evaluating on-going biological control with *Steinernema scapterisci* (Nguyen and Smart) nematodes. Seven ~4-ha pastures were selected from five county sites for the survey. These consisted of one mole cricket-infested bahiagrass pasture each from two ranches in Polk county and from one ranch each in Manatee and Pasco counties. The rest were two renovated and uninfested pastures located at the Range Cattle Research and Education Center, Ona, in Hardee county and a third in Desoto county. Six linear pitfall traps (each 12.2 m total) were installed on equal subdivisions (0.67 ha) of each of the seven pastures in July 1997 and labeled 1 to 6 at each site according to a visually-determined decreasing slope of terrain. Traps were cleaned weekly from the time of installation through December 1999, and the total weekly-captures per trap of tawny, *S. vicinus* (Scudder) and southern, *S. borellii*, (Giglio-Tos) mole crickets were recorded along with weekly rainfall for each site. The mean, weekly mole cricket capture on heavily-infested bahiagrass pastures increased exponentially over time beginning with the early summer rains. Mean weekly-count on these pastures peaked at 20-60 juveniles per trap, depending on site, in June-July and then declined sharply through September and October as mole crickets matured. The annual mean weekly-capture on heavily-infested pastures was 10 to 12 juveniles per trap. There was very little surface activity by overwintering adult mole crickets during December and January. Mean weekly-capture on uninfested new bahiagrass pasture was erratic and usually less than 2 juveniles per trap. The data suggest that peak weekly mole cricket pitfall trap captures between June and August in excess of 20 juveniles per trap and a total seasonal capture in excess of 43 m^{-1} of trap were indicative of a serious infestation problem.

Key Words: *Scapteriscus vicinus*, *Scapteriscus borellii*, *Paspalum notatum*, Linear pitfall trap, Pasture grasses

Resumen

Se necesita la historia de la actividad de plagas de los grillotopos (*Scapteriscus* spp.) (Orthoptera: Gryllotalpidae) en pastizales de grama de bahia (*Paspalum notatum* Fluegge) para proveer los datos iniciales para evaluar el control biológico en marcha con los nemátodos, *Steinernema scapterisci* (Nguyen and Smart). Siete pastizales de ~4-ha fueron seleccionadas de sitios en cinco condados para el sondeo. Estas consistian en una pastizal de grama de bahia infestada con grillotopos cada una de dos haciendas el condado de Polk y de una hacienda en cada uno de los condados de Manatee y de Pasco. Los restos fueron dos pastizales renovados y no infestados situados en el Range Cattle Research and Education Center, Ona (Centro de Investigación y Educación de Ganado de Rangos) en el condado de Hardee y una tercera en el condado de De Soto. Seis trampas de "pitfall" (trampas donde la presa cae en un hoyo en el suelo) en linea (cada 12.2 m total) fueron instaladas en subdivisiones iguales (de 0.67 ha) en cada una de los siete pastizales en julio de 1997 y marcadas 1 a 6 en cada sitio según la inclinación de terreno visualmente determinada. Se limpiaron las trampas semanalmente desde el tiempo de instalación hasta el diciembre de 1999, y se registrarón el número total del grillotopo aleonado, *S. vicinus* (Scudder) y del grillotopo sureño, *S. borellii*, (Giglio-Tos) capturados por trampa por cada semana junto con la cantidad de lluvia que cayó en cada sitio por semana. El promedio del número de los grillotopos capturados cada semana en pastizales de grama de bahia infestadas altamente aumentó exponencialmente sobre el tiempo empezando con las lluvias en el principio de verano. El promedio del número capturado semanalmente en estas pasturas llegó a lo mas alta de 20-60 juveniles por tampa, dependiendo del sitio, en junio-julio y luego bajó agudamente en septiembre y octubre cuando los grillotopos maduraron. El promedio anual del número capturado en pasturas altamente infestadas fué 10 a 12 juveniles por trampa. Habia muy poca actividad sobre la superficie por

adultos de grillotopos invernando durante diciembre y enero. El promedio del número capturado semanalmente en pastizales de grama de bahia no infestadas fué érratico y usualmente menos de 2 juveniles por trampa. Estos datos sugieren que el número más alto de grillotopos capturados semanalmente en las trampas "pitfall" entre junio y agosto en exceso de 20 juveniles por trampa y una cantidad en exceso de 43 m^{-1} por trampa por toda la estación fueron indicativos de un problema de infestación seria.

Adventive mole crickets *Scapteriscus* spp. (Scudder) cause serious damage to pasture, lawn and crops in Florida. It is estimated from a survey (South Florida Beef and Forage Extension Program 1999) that nearly $45 million-revenue is lost annually to cattle producers in south central Florida as a result of reduction in hay and forage production as a result of mole cricket damage and an extra $10 millon/year spent on pasture renovation.

All three pest mole crickets found in Florida: tawny, *S. vicinus* (Scudder), southern, *S. borellii* (Giglio-Tos) and short-winged, *S. abbreviatus* (Scudder); were inadvertently introduced from South America in ship's ballast into ports of Georgia, South Carolina, Alabama and Florida in the early 1900s (Walker & Nickle 1981). From these points of arrival, the tawny and southern mole crickets spread westwards and southwards, and by 1960 had covered and become serious pests throughout Florida (Walker & Nickle 1981). Due to its inability to fly, the short-winged is largely restricted to point of introduction in coastal areas.

Mole crickets spend nearly all their year-long life cycle underground (Walker 1984), which makes population sampling very difficult. Eggs are laid in clutches in underground chambers. Nymphs tunnel to the surface and feed in the upper soil and litter. Juveniles and adults make and occupy extensive gallery and tunnel systems. In south and central Florida, tawny mole cricket has one generation per year, but the southern mole cricket has two generations annually (Walker 1984). It is only during their peak mating flights in early-spring and to a lesser extent in the fall that pest mole crickets are conspicuous to the casual observer.

Mole cricket damage to pasture and turfgrass is principally due to feeding by tawny mole crickets (Walker & Dong 1982; Hudson 1984). At night, mole crickets usually leave their tunnels to bite off stems and leaves, which are dragged into their burrows to be eaten. Roots are eaten at any time from within tunnels. Mechanical damage to plants is caused by the tunneling activity of mole crickets and this is the principal detrimental effect of southern mole crickets on pasture. Damage in pasture first appears in yellow patches which die and turn brown. In areas of high mole cricket population density, the surface 20 to 25-cm soil layer is honeycombed with numerous galleries and the ground feels spongy when stepped on. Heavily-damaged pasture has virtually no root system and is easily pulled from the soil by cattle or foot traffic in a pasture.

The most direct way to evaluate classical biocontrol agents is to compare the population levels of target species before and after establishment of natural enemies. Three basic sampling techniques for monitoring mole crickets have been described (Hudson 1988) although none has overcome the basic obstacle of showing good correlation with true population density. These sampling techniques are sound traps for flying adults (Walker 1982), linear pitfall traps for monitoring the activity of immature mole crickets (Lawrence 1982) and soil flushing for both juveniles and adults (Short & Koehler 1979). More than 20 yr data have been collected around suburban Gainesville and Bradenton on adult pest mole crickets' flight with sound traps (Walker et al. 1995). However, adult mole crickets can fly over long distances, and sound trap captures may not reflect the level of mole cricket infestation on specific ranches. Hudson (1989) developed an equation for soap flushing from repeated sampling that predicted mole cricket population estimates within 25% of the true population, however, soap detergent is lethal to *S. scapterisci* nematodes (Grover Smart, Jr., pers. comm.). Lawrence (1983) used linear pitfall traps to monitor the activity of juvenile mole crickets on pasture. Data on seasonal activity of mole crickets in Florida pastures are lacking.

This study was designed to use permanently set pitfall traps to monitor the long-term seasonal abundance of immature mole crickets on pasture in relation to rainfall and pasture damage. Abundance histories developed on specific ranches was to be used as baseline information for future evaluation of biocontrol with nematodes.

Materials and Methods

The survey was conducted on five ranches in south-central Florida and the Range Cattle Research and education Center, Univ. of Florida, following a severe mole cricket outbreak on pastures in central Florida in 1996. These sites represented two extremes of initial mole cricket infestation. In July 1997, six pitfall traps were installed on one 4-ha bahiagrass pasture at each of two ranches (A. D. Combee and George Clark) which had heavy mole cricket damage in the Green Swamp area of Polk county and one similar bahiagrass pasture each in Manatee (Harlee ranch) and Pasco (Mary Nutt ranch) counties. Six traps were also installed on two 4-ha, renovated

pastures which appeared to be lightly infested with mole crickets at the Range Cattle REC, Ona, in Hardee county and another similar pasture in Desoto county.

The 4-ha bahiagrass pasture at each site was divided into six equal blocks (reps) each installed with one trap. Traps at each site were labeled 1 to 6 in decreasing slope of terrain and were cleaned weekly from July 1997 through December 1999. At cleaning, weekly-captured tawny and southern mole crickets in each trap were counted together. Body decomposition of nymphs during summer rainfall made differentiation between remaining tiny heads of tawny and southern mole crickets very difficult and unreliable. However, the heads were resistant to decomposition and used as markers for the weekly counts whenever body decomposition occurred. Development of immature mole crickets was monitored at one site in the Green Swamp area by measuring the length of the pronotum of 20 trapped mole crickets monthly from June 1998 to April 1999. Amount of weekly rainfall was recorded for the two Green Swamp sites in Polk county, the Manatee, and the Pasco sites. Bahiagrass pasture on each site was rated as to percentage green, yellow, dead or brown, bare ground, and weed cover, every spring using a subdivided m^2 quadrat. The quadrat had 100 divisions, each representing a percentage unit, and was thrown randomly to twenty-four locations (4 on each subdivision) on the 4-ha pasture.

Data on weekly trapped mole crickets were subjected to statistical analysis of variance (SAS 1999) with site as main plot and year and week as split and split-split plots in time, respectively, and traps as replicated blocks. Due to significant site by week ($P < 0.0008$) and year by week ($P < 0.0076$) interactions, weekly abundances of trapped mole crickets were fitted to week of the year, separately for each site, using SigmaPlot regression software (SPSS, Inc. 1997) that maximized regression R^2 and minimized standard error (SE). Ratings of pasture condition were analyzed as a split plot with site as main plot, year as split plot in time and pasture subdivision as replicates.

Results

The analysis of variance of weekly trapped immature mole cricket numbers on bahiagrass pastures from July 1997 to June 1999 is shown in Table 1. There was a distinct migration of pasture mole crickets from low-lying, inundated soils to drained soils at the peak of summer rains and vice versa during the dry spring period. This resulted in large trap location and trap location × week effect. As expected, trapped mole cricket numbers also varied depending on site of bahiagrass pasture, year and week of the year. The site × week, year × week interactions were significant and the site × year × week interaction approached significance ($P < 0.10$).

The 3-yr mean weekly trapped mole crickets ranged from 10.1 to 12.4/trap for the heavily mole cricket-infested pastures in the Green Swamp in Polk county, and the sites in Pasco and Manatee counties (Table 2). From 0.7 to 1.7 mole crickets/trap were found in the lightly-infested bahiagrass pastures in Desoto and Hardee counties.

Level of mole cricket infestation was highly correlated with pasture damage (r = 0.89). Sites where seasonal mean weekly mole cricket trap captures >10, such as Combee, Clark, Nutt, and Harlee ranches, had severe pasture damage (yellow + dead/weeds) ranging from 49 to 72% (Table 2). Conversely, the lightly-infested mole cricket sites including pastures at the Range Cattle REC and Desoto county stayed green in spring and showed little sward damage.

Mean weekly capture of nymph and adult pest mole crickets on damaged bahiagrass pastures within the year was best described by an exponential curve (Gaussian 3 Parameters) (Figs. 1-4). Most mole cricket eggs normally hatch in May and June (Walker 1984), and the number of trapped nymphs on pasture reached a peak after

Table 1. Sources of variation, degrees of freedom, F-test value, and the probability of a greater F-value for weekly distribution of pest mole crickets on bahiagrass pastures in south-central Florida.

Variable	df	F	Pr > F
Trap	5	6.86	0.0001
Site	6	3.08	0.0255
Site × Trap (Error a)	30		
Year	2	7.44	0.0001
Site × Year	12	0.64	0.8979
Site × Year × Trap (Error b)	60		
Week	51	3.72	0.0001
Site × Week	306	1.84	0.0008
Year × Week	102	1.72	0.0075
Site × Year × Week	612	0.86	0.0998
MSE (Error c)	4105		

TABLE 2. THE EFFECT OF BAHIAGRASS PASTURE SITE ON 3-YR MEAN WEEKLY PEST MOLE CRICKET COUNT/TRAP AND CORRESPONDING PASTURE DAMAGE.

County	Ranch	Weekly mole cricket count/trap	Damage estimate		
			Green	Yellow	Dead/weeds
		No.	% cover		
Polk	A. D. Combee	10.1	45	4	51
Polk	George Clark	12.4	50	12	38
Manatee	Harlee Farm	11.2	28	10	62
Pasco	Mary Nutt	11.0	51	37	12
Hardee	RCREC-71A[1]	0.7	98	1.5	0.5
Hardee	RCREC-87[1]	1.7	85	5	10
Desoto	Steven Houk	1.6	97	2	1.0
	LSD $P = 0.05$	5.7	12	8	10

[1]Range Cattle Research and Education Center, pastures 71A and 87.

the first major summer rainfall in June or July. For the 2.5 yr study, week 30 (X_0) had the highest mean peak weekly mole cricket trap count ('a' value) of 23 on Combee ranch (Fig. 1), week 25 of 26 peak trap count on Clark ranch (Fig. 2), and week 25 of 40 peak trap count on Nutt ranch (Fig. 3). On all these severely-damaged fields, there was at least one weekly-spike episode that

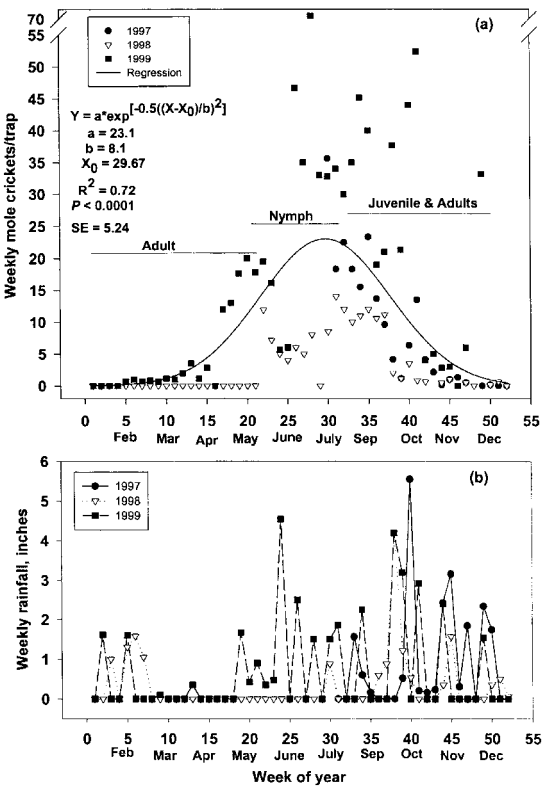

Fig. 1. Seasonal distribution (1997-1999) of mean weekly pitfall trap captures of immature *Scapteriscus* mole crickets (a) in relation to weekly rainfall pattern (b) on bahiagrass pasture at A.D. Combee Ranch in Polk county.

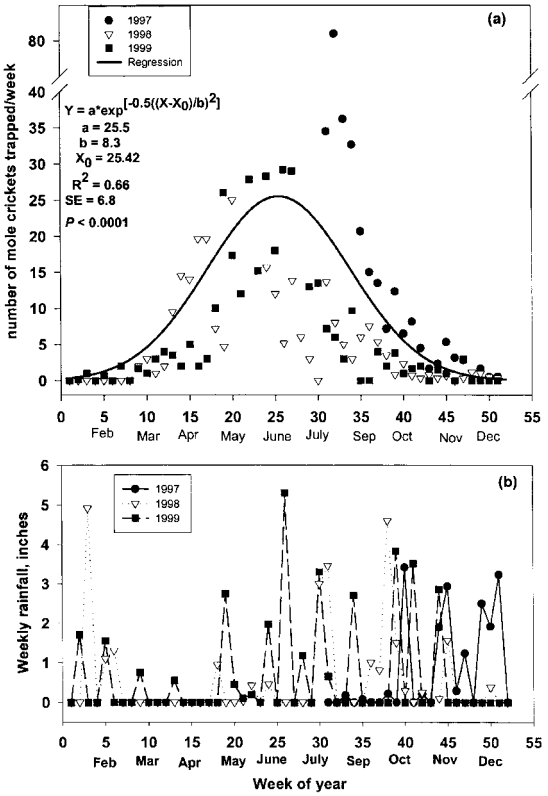

Fig. 2. Seasonal distribution (1997-1999) of mean weekly pitfall trap captures of immature *Scapteriscus* mole crickets (a) in relation to weekly rainfall pattern (b) on bahiagrass pasture at George Clark Ranch in Polk county.

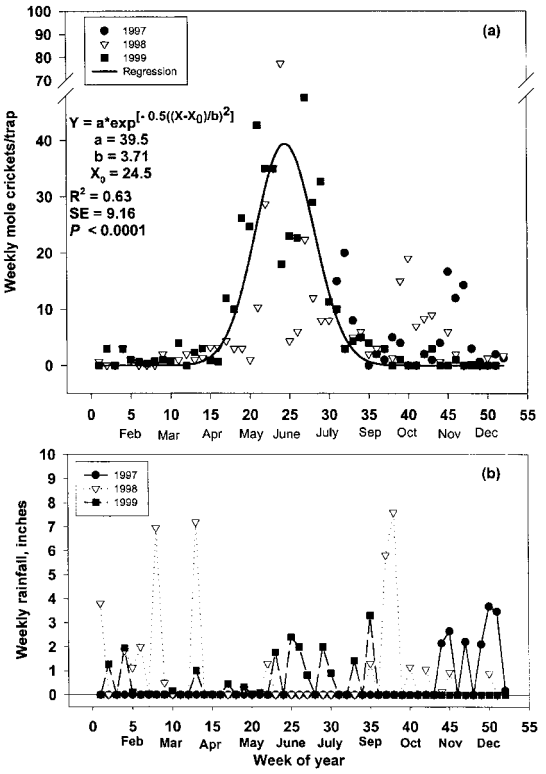

Fig. 3. Seasonal distribution (1997-1999) of mean weekly pitfall trap captures of immature *Scapteriscus* mole crickets (a) in relation to weekly rainfall pattern (b) on bahiagrass pasture at Mary Nutt Ranch in Pasco county.

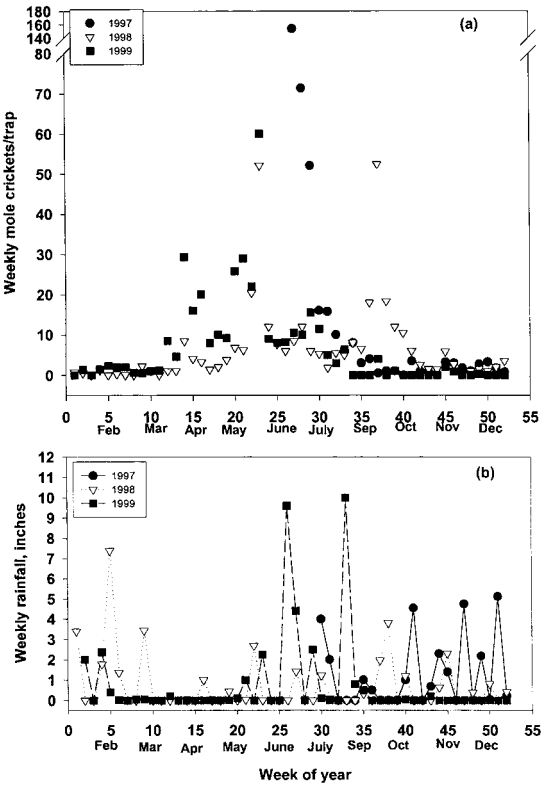

Fig. 4. Seasonal distribution (1997-1999) of mean weekly pitfall trap captures of immature *Scapteriscus* mole crickets (a) in relation to weekly rainfall pattern (b) on bahiagrass pasture at Harlee Ranch in Manatee county. Year × week interaction $P < 0.05$.

exceeded 50 nymphs/trap during the 2.5-yr monitoring. On Harlee ranch, also a heavily infested pasture, we experienced variable patterns of weekly nymph abundance over the years and a single exponential curve did not provide a good fit across years (Fig. 4). A weekly peak >100 nymphs/trap occurred in early July of 1997. A bimodal peak (June and September) averaging 45 nymphs/trap was observed in 1998, and a weekly peak of only 20 nymphs/trap in 1999 (Fig. 4). Weekly spikes of trapped nymphs on heavily infested pastures usually coincided with rainfall that saturated the soil early in the summer, forcing the nymphs to relocate within a pasture. Subsequent heavy rainfall within the season was not generally accompanied by similar hikes in nymph activity because they had already dispersed to higher grounds that were less likely to become saturated. There was also a lack of fit of trapped mole cricket data to week of sampling on the lightly-infested pastures in Hardee sites (Fig. 5) and mole cricket infestation on DeSoto site was low (‘1.6 nymphs/trap/week) but uniform throughout the year (plot not shown).

The mean pronotal length of 20 pitfall trap-captured mole crickets was 3.1 ± 0.3 mm in June; 4.8 ± 0.2 mm in July; 5.2 ± 0.3 in August; 5.8 ± 0.4 mm in September; 6.9 ± 0.5 mm in October; 7.3 ± 7 in November; and 11.6 ± 1.0 in April. When discernible, the ratio of numbers of tawny:southern mole crickets on pasture was approximately 3:1 but there was no difference in pronotal length between tawny and southern mole crickets. The increase in pronotal length and standard deviation of pronotal length over time was due to an increasing adult component in the population later in the season.

Discussion

The extent of damage observed on pastures was dependent on the level of pest mole cricket infestation. A seasonal mean-weekly mole cricket nymph trap capture >10 was associated with more than 50% pasture damage. A mean-weekly trap capture of 10 mole crickets amounts to 520 mole crickets per trap yearly. A mean-weekly trap capture of 1.7 mole crickets or 88 mole crickets per year was associated with moderate pasture damage (15%).

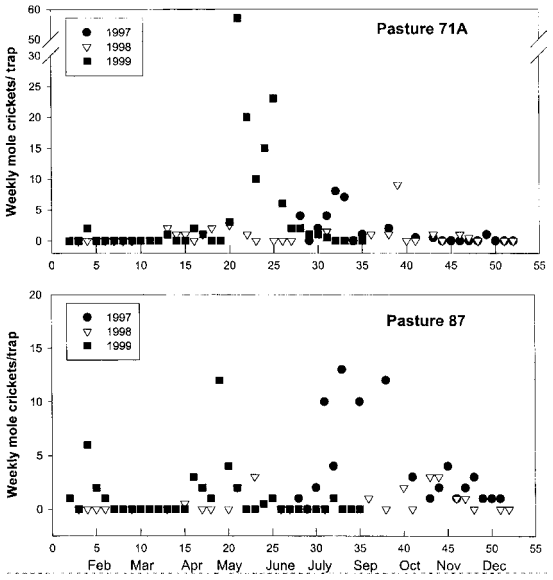

Fig. 5. Seasonal distribution (1997-1999) of mean weekly pitfall trap captures of immature *Scapteriscus* mole crickets on bahiagrass for Pastures 71A and 87 at the Range Cattle REC, Ona, in Hardee county.

Each trap had a total length of 12.2 m which suggests that the seasonal damage threshold falls somewhere between 7 and 43 mole crickets m^{-1} on bahiagrass pasture. In a 1-year study (1 May 1982 to 30 April 1983), Lawrence (1983) installed three linear pitfall traps (5.5 m total length) on bahiagrass turf in Palm Beach within 4.6 m of a sound trap station (Walker 1982) and captured 609 immature mole crickets of which 89 were *S. borellii*, 139 were *S. vicinus* and 381 were *S. abbreviatus.* Within the same 12 month period, 13,496 adult *S. borellii* and 197 *S. vicinus* were captured in the sound station. It is known that many crickets attracted to sound callers miss the sound trap, therefore, Lawrence's (1983) total capture of 111 *Scapteriscus* nymphs m^{-1} of pitfall trap could have been inflated by egg deposition in the vicinity from adult females which missed the sound traps. We did not encounter any *S. abbreviatus* in our study on ranches in central Florida probably because that species was restricted to coastal areas where it was first introduced because it cannot fly. Similar to our data, 85% of Lawrence's (1983) mole cricket nymphs were captured between June and August, and the nymphs increased in pronotal length from 2 to 9 mm between June and April.

Mole cricket buildup on Hardee and DeSoto sites following pasture renovation was slow. Results seem to agree with the general producers' perception that it takes >3 yr after a successful pasture renovation before mole cricket populations in bahiagrass fields build up to damaging thresholds.

Decline of mole cricket pitfall captures in late summer and fall has been attributed to marked differences between nymph and adult mole cricket behavior (Hudson 1989) and also to the action by generalist native natural enemies (Hudson et al. 1988). Available evidence (Hudson 1985; Hudson & Shaw 1987) suggests that nymphs are largely nomadic, with no "home" burrow, and so are more likely to seek escape on the surface whereas adults often have an established and deep burrow system into which they retreat rather than coming to the surface. Adult females in the fall tend to dig a permanent burrow system and stay there, with little apparent foraging. Males are more active on the surface but not as active as nymphs and they also dig extensive burrow systems (Nickerson et al. 1979).

Both the natural decline in surface activity and the action by generalist natural enemies have been inadequate to prevent heavy damage in pastures and turf during fall, for which reason a program was begun to import South American specialist natural enemies (Sailer 1984). Four specialist natural enemies have been imported to Florida, three of them released and established, and their future in integrated pest management of pest mole crickets has been outlined (Frank & Parkman 1999). The nematode, *S. scapterisci* Nguyen & Smart (1990), received research emphasis in the late 1980s, attracted attention from industry as a biopesticide, and perhaps appeals to most ranchers because it can be purchased and applied as a pesticide (a method familiar to them). Our preliminary analysis did not show nematode infection of mole crickets trapped in any of the test sites. Other biocontrol agents such as *Ormia depleta* Wiedemann and *Larra bicolor* F. may yet prove to be more effective and less costly, but data on their efficacy are incomplete, and methods for management of their field populations are inadequately researched because of lack of funds.

Development of mole cricket seasonal activity history on bahiagrass pasture is critical for evaluation of a successful outcome of any biocontrol agent. Spikes of nymphs exceeding 100 per pitfall trap may be captured following the early summer rains, but a mean seasonal capture >10 nymphs m^{-1} of trap may indicate the start of a mole cricket infestation problem and may represent a working threshold for future studies.

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COMPARISON OF BIODEGRADABLE, PLASTIC AND WOODEN IMIDACLOPRID-TREATED SPHERES FOR CONTROL OF *RHAGOLETIS MENDAX* (DIPTERA: TEPHRITIDAE) FLIES

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ABSTRACT

In experiments comparing biodegradable, plastic and wooden imidacloprid-treated spheres for control of *Rhagoletis mendax* Curran, the mean number of flies caught on plexiglas panes below each sphere type was not significantly different for the entire season. However, the mean time spent by *R. mendax* flies alighting on biodegradable imidacloprid-treated spheres was significantly greater (2.6×) than plastic imidacloprid-treated spheres. During 2001, significantly fewer larvae were found in blueberries harvested from bushes that had wooden imidacloprid-treated spheres hung within the canopy compared with bushes where biodegradable and plastic imidacloprid-treated spheres were deployed. There was no significant difference between the number of larvae found in berries picked from bushes where biodegradable or plastic spheres were deployed. All imidacloprid-treated sphere treatments were found to significantly reduce blueberry maggot larval infestation in fruit compared with the control.

Key Words: attractant, imidacloprid-treated sphere, blueberry maggot

RESUMEN

En experimentos comparando las esferas de plástico y de madera biodegradable y tratadas con imidacloprid para el control de *Rhagoletis mendax* Curran, el promedio del número de las moscas atrapadas sobre la superficie de "plexiglas" debajo cada clase de esfera no fué significativamente diferente para la estación completa. No obstante, el promedio del tiempo pasado por mosca de *R. mendax* encima de las esferas biogradables tratadas con imidacloprid fué significativamente mayor (2.6 veces) que en las esferas plasticas tratadas con imidacloprid. Durante 2001, fueron significativamente encontradas menos larvas sobre las moras (*Vaccinium* sp.) cosechadas de arbustos que tenian las esferas de madera tratadas con imidacloprid colgadas dentro del dosel comparados con arbustos donde pusieron esferas de plástico biodegradable y tratadas con imidacloprid. No habia una diferencia significativa entre el número de larvas encontradas en la frutas cortadas de los arbustos donde habian puestas las esferas de plástico biodegradable y tratadas con imidacloprid. Se encontraron que todos los tratamientos de las esferas tratadas con imidacloprid redujieron significativamente la infestación de larvas en las frutas comparados con el control.

The potential for using a lure and toxicant system to control fruit flies has been examined by several researchers. Hanotakis et al. (1991) combined a food attractant, a phagostimulant, a male sex pheromone, a female aggregation pheromone, a hygroscopic substance (glycerin), and two insecticides (deltamethrin and dichlorvos) with a trap to control the olive fruit fly, *Bactrocera oleae* (Gmelin). Duan and Prokopy (1995) and Hu et al. (2000) tested dimethoate, abamectin, phloxine B, diazinon, imidacloprid, azinphosmethyl, methomyl, tralomethrin, malathion, fenvalerate, and carbaryl on wooden spheres and found that only dimethoate, malathion and imidacloprid were viable candidates for incorporation into spheres to suppress apple maggot, *Rhagoletis pomonella* (Walsh), activity. Dimethoate, malathion, and imidacloprid did not reduce the time of visitation by *R. pomonella* flies on treated spheres in field cage studies.

Recently, Ayyappath et al. (2000) evaluated thiamethoxam at 2-4% AI in sugar/starch spheres and found this insecticide to be significantly less effective than spheres treated with 2% AI imidacloprid. Wright et al. (1999) determined that regardless of trap design and pesticide incorporation, several conditions must exist for spheres to become a viable alternative for control of *Rhagoletis* flies. Spheres must be: 1) easy and safe to deploy, 2) as effective as insecticide sprays, 3) able to endure throughout the growing season, and 4) maintain fly-killing power with a very low dose of toxicant.

A recent trap design is a biodegradable sphere consisting of water, gelatinized corn flour, corn syrup, sugar, cayenne pepper, and sorbic acid (Liburd et al. 1999; Stelinski & Liburd 2001). The biodegradable sphere is coated with a mixture of 70% paint, 20% sucrose solution (wt:vol), 4% imidacloprid (AI), and 6% water. Biodegradable

spheres were developed as alternatives to broad-spectrum insecticides for management of key *Rhagoletis* spp. in the northeastern United States. The benefits of using insecticide-treated spheres include the reduction of pesticide residues on crops as well as reduced environmental and worker hazards.

The purpose of this study was to compare biodegradable, plastic, and wooden imidacloprid-treated spheres to determine the most efficacious sphere type for preventing blueberry maggot injury. All previous trap designs, with the exception of the biodegradable sphere, had focused on using wooden spheres brush painted with enamel paint mixed with an insecticide. Using a plastic sphere, either dipping it into an insecticide/sugar solution or coating it with a mixture of paint and insecticide presents a third alternative to previous designs.

Materials and Methods

Research plots were located in Rhode Island and Michigan. In Rhode Island plots were located at two locations during 2000, a 0.5 ha highbush blueberry, *Vaccinium corymbosum* L., planting of 'Patriot', 'Blueray', and 'Jersey' located in North Kingstown and a 2 ha planting of 'Berkley' and 'Collins' located in West Kingston. In 2001, research was conducted at a 0.3 ha planting of 6 cultivars; 'Bluecrop', 'Bluetta', 'Darrow', 'Earliblue', 'Herbert' and 'Lateblue' in Kingston, RI and at a 2 ha planting of 'Jersey' located in Holland, MI.

Sphere preparation (2000)

Biodegradable spheres were obtained from the USDA, National Center for Agricultural Utilization Research Laboratory in Peoria, Illinois and prepared as described in Liburd et al. (1999). Spheres were brush painted with a mixture containing 70% enamel paint (Shamrock Green 197A111, ACE Hardware, Kensington, IL.), 20% (wt:vol) sucrose solution, 2% (AI) imidacloprid (Provado 1.6 F, Bayer, Kansas City, MO), and 8% water.

Plastic spheres (Great Lakes IPM, Vestaburg, MI) were dipped in a solution containing 946 ml water, 28 g of Merit 75 WP (imidacloprid) (Bayer, Kansas City, MO), 189 ml of 20% sucrose solution (wt:vol), and 22 ml (2 ml of product in 20 ml water) finished additive of Turbo spreader (Bonide, Yorkville, NY). This mixture represents 81.6% water, 2.4% Merit 75WP (1.8% AI imidacloprid), and 16% (wt:vol) sucrose solution. Spheres were dipped a total of three times during the growing season.

2000

Three treatments were evaluated in two highbush blueberry plantings for control of *R. mendax* in a completely randomized block design with four replicates. Each block consisted of ten 9-cm diameter green biodegradable imidacloprid-treated spheres (treatment 1), ten 9-cm diameter green plastic imidacloprid-treated spheres (treatment 2), and a section of the block consisting of 30 bushes was left untreated (treatment 3, control). Spheres, approximately one per three bushes, were hung about 15-cm from the uppermost bush, which is the most effective position (Liburd et al. 2000), and baited with ammonium acetate (1 g in 4 ml of water) in a 5 ml scintillation vial (National Diagnostics, Atlanta, GA). A 45 cm × 45 cm square of plexiglas spray-coated with Tangletrap (The Tanglefoot Co., Grand Rapids, MI) was hung 30 cm beneath each of the imidacloprid-treated spheres and supported by four tie-wires (Liburd et al. 1999).

During each sampling period, *R. mendax* flies that landed on treated spheres were observed for 30 minutes; a total of 54 flies were observed. *R. mendax* flies captured on plexiglas panes were counted and removed twice weekly. In addition to monitoring fly populations with Plexiglas panes, *R. mendax* fly populations were also monitored twice weekly using 9-cm diameter unbaited green plastic spheres coated with Tangletrap. Towards the end of the season, an 8-liter sample of 'Patriot' and 'Blueray' was taken on July 13 and 'Blueray' and 'Jersey' taken on July 27 from North Kingstown, RI and placed on screens (0.5 cm mesh) over clear plastic containers to determine the number of maggots in fruit (Liburd et al. 1998). The number of maggots collected into the containers was counted twice a week to determine the effectiveness of the sphere treatments. Fruit was not sampled from West Kingston, RI because deer had damaged the majority of the biodegradable spheres.

Sphere preparation (2001)

During 2001, sphere preparation methods differed from those used in 2000 because additional research data were available on the deployment of insecticide-treated spheres.

Biodegradable imidacloprid-treated spheres were prepared as described in 2000. However, the active ingredient (AI) was increased to 4% because Stelinski et al. (2001) had shown that the effectiveness of field-exposed imidacloprid-treated spheres with 2% AI was significantly reduced over a 12 wk period whereas spheres treated with 4% AI were not significantly affected.

Plastic imidacloprid-treated spheres were first painted with a mixture of 26 ml Provado 1.6F (4% AI imidacloprid) (Bayer, Kansas City, MO), 87 ml 'Bell Pepper' paint (Pittsburgh Paints, Pittsburgh, PA) and 20 ml sucrose solution (5.5 g per 20 ml water = ca. 4.8% of the total mixture). In addition, a newly developed sucrose cap (Prokopy et al. unpublished data) was attached to the spheres to act as a feeding stimulant.

2001

In Rhode Island, the same three treatments (biodegradable and plastic imidacloprid-treated spheres and control) evaluated in 2000 were re-evaluated in 2001. In Michigan, a fourth treatment, wooden imidacloprid-treated spheres, was included in the experimental design. Wooden imidacloprid-treated spheres (9-cm) were brush painted with a mixture of DevFlex latex green paint (ICI Paints, Cleveland, OH) (70%), sucrose feeding stimulant (20%), water (6%), and imidacloprid (4% AI). Like plastic spheres, wooden spheres had the sucrose cap attached to act as a feeding stimulant.

The experimental design was similar to 2000 and consisted of randomized block with four replicates. The placement and position within the canopy of imidacloprid-treated spheres were the same as 2000. However, spheres were baited with polycon dispensers containing 5 g of ammonium carbonate (Great Lakes IPM, Vestaburg, MI). The dispensers were attached to the strings used for hanging spheres. Flies were monitored using the same Plexiglas pane system used in 2000.

In Michigan, four samples of 100 berries per replicate (totaling 400 berries per treatment) were taken July 31 and August 1, 3, and 8. Berries were then placed over 0.5 cm mesh hardware cloth to allow larvae to exit the fruit and drop into containers filled with vermiculite (Liburd et al. 1998). The vermiculite was then sifted and blueberry maggot fly puparia were collected and counted to quantify fruit infestation.

Statistical Analysis

Data were analyzed by analysis of variance. (SAS Institute 1989).

RESULTS

2000

The population of *R. mendax* flies at the North Kingstown, RI site was small, and captures on plexiglas panes for biodegradable and plastic imidacloprid-treated spheres were not significantly different, except on July 18th (Fig. 1). However, Plexiglas panes placed under biodegradable spheres consistently captured more flies than panes placed beneath plastic spheres. The time spent by *R. mendax* flies on biodegradable imidacloprid-treated spheres (62.6 ± 12.0 sec) was significantly greater ($F = 32.5$, df = 26, 53; $P < 0.01$) than the time spent by flies on dipped plastic spheres (24.2 ± 24.2 sec).

Data collected using 9-cm diameter, unbaited, green plastic sticky spheres coated with Tangletrap indicated that peak flight activity occurred on July 4th. No maggots were found in 32 liters of berries harvested on July 13th and 27th from any of the treatment blocks including the control. The data at the West Kingston, RI site could not be analyzed due to the high incidence of deer damage.

2001

In Rhode Island, the mean number of flies collected on plexiglas panes below plastic (35.6 ±

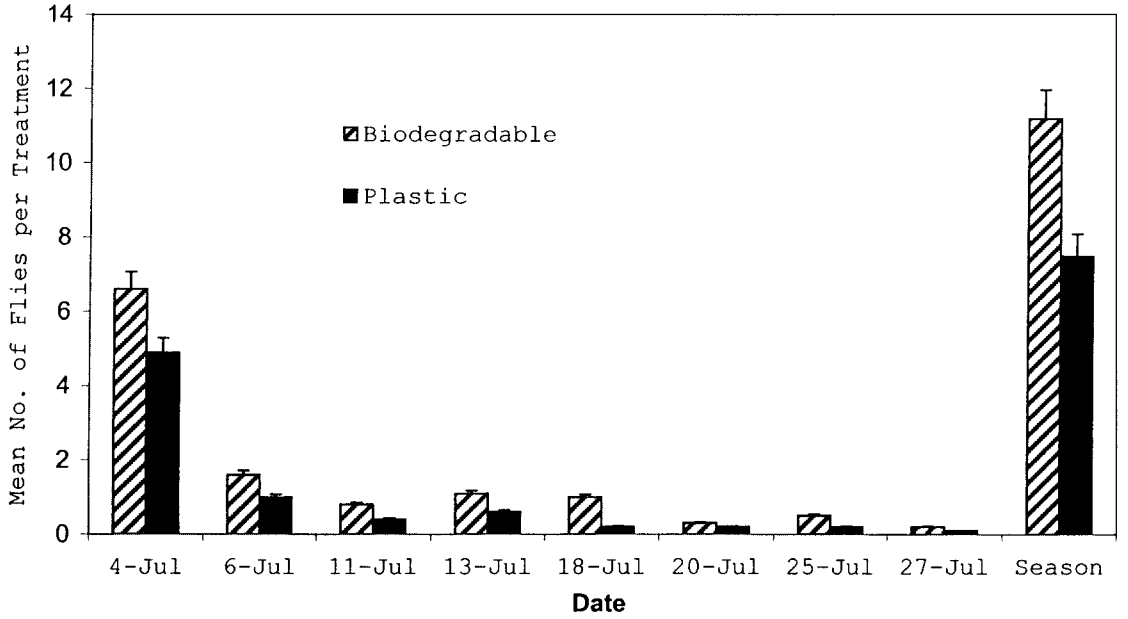

Fig. 1. Mean number of *R. mendax* flies trapped on plexiglas panes beneath imidacloprid-treated biodegradable and plastic spheres, North Kingston, RI July 4-27, 2000.

16.0) and biodegradable (24.6 ± 11.0) imidacloprid-treated spheres was not significantly different for the entire season. Similarly, in Michigan the mean number of flies collected on Plexiglas panes below biodegradable (33.8 ± 2.98), wooden (31.3 ± 4.71), and plastic (26.0 ± 9.06) spheres was not significantly different for the entire season. Again, plexiglas panes placed below biodegradable spheres consistently captured more *R. mendax* flies than plastic and wooden spheres.

In our fruit infestation counts, significantly fewer ($F = 24.63$, df = 3,6, $P < 0.01$) larvae were collected from berries that had wooden spheres deployed in blocks compared with plastic and biodegradable spheres (Fig. 2). Overall, the mean number of larvae found in berries treated with biodegradable, plastic, or wooden spheres was significantly lower ($F = 24.63$, df = 3,6, $P < 0.01$) than untreated checks. Berries collected from untreated (control) plots had 1.8 times as many larvae compared with other treated plots (Fig. 2). Six biodegradable imidacloprid-treated spheres were lost to deer feeding during the 6 weeks of experimentation in Michigan. Peak flight activity for *R. mendax* occurred on July 24 as measured with yellow unbaited sticky boards.

Discussion

Experiments comparing the effectiveness of biodegradable, wooden, and plastic imidacloprid-treated spheres showed no significant differences in the number of flies trapped on Plexiglas panes. This is the first study showing the effectiveness of plastic imidacloprid-treated spheres for suppression of *R. mendax*. Previous studies have focused on the efficacy of wooden and biodegradable insecticide-treated spheres. Currently, wooden spheres are not commercially available. Also, production of wooden and biodegradable spheres may be prohibitive since the cost may range between $2-4 per sphere for either sphere type. In blueberries, depending on infestation of *R. mendax*, it may take as many as 100 spheres per hectare for effective control.

The variation in sphere preparation throughout 2000 and 2001 was done to optimize insecticide concentration and formulation as well as to further develop the feeding stimulant system so that flies will alight for longer time on treated spheres. Our data showed that flies spent much longer time on biodegradable imidacloprid-treated spheres compared with plastic imidacloprid-treated spheres. However, the fact that larval infestation was not significantly affected between sphere types may indicate that the duration of stay on treated spheres to deliver a lethal dose may not be as important as previously thought. Insecticide compatibility with treated spheres and susceptibility of the insect may be the key factors regulating the effectiveness of insecticide-treated spheres.

Because data from our green sticky sphere monitoring traps indicated that *R. mendax* flies were active throughout the season and flies were trapped continuously with our Plexiglas trapping device, it was rather surprising that no larvae were found in treated and untreated plots at our North Kingston, Rhode Island site. However, because the plots were relatively small, it is possible

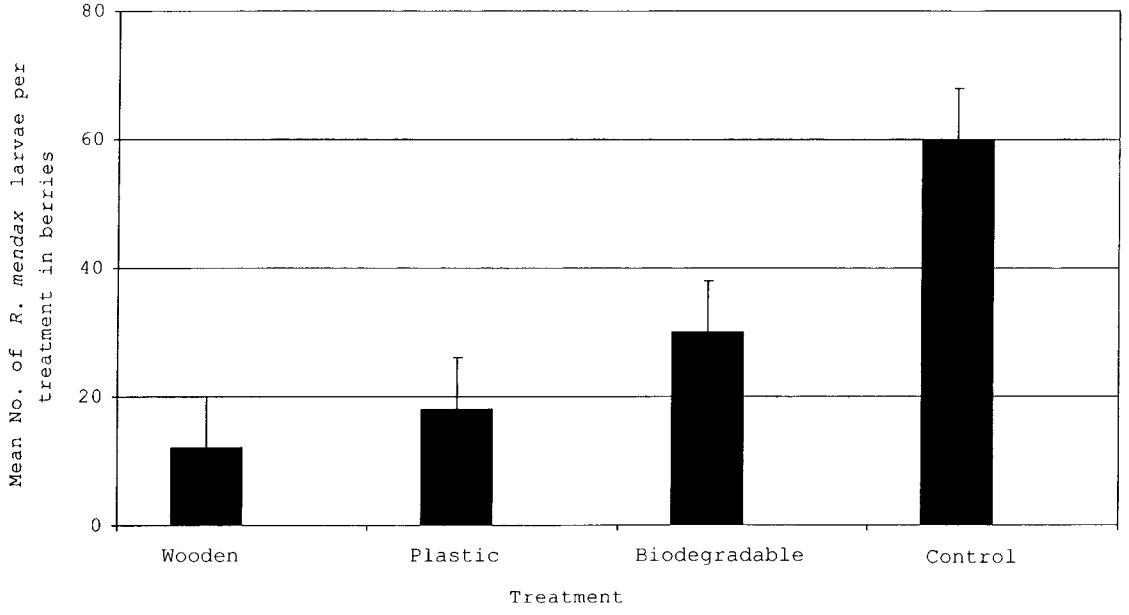

Fig. 2. Mean number of maggots in four samples of 100 berries, Holland, MI (2001).

that the ammonium acetate attractant used for baiting imidacloprid-treated spheres may have attracted flies from treated and untreated areas resulting in a high mortality and subsequently preventing infestation in both treated and untreated plots. Liburd et al. (1999) also found that ammonium lures were effective in attracting *R. mendax* flies from within a 5 m radius to insecticide-treated sphere traps.

The biodegradable imidacloprid-treated spheres used in our study may be more appealing to growers than the plastic spheres used in 2000. Biodegradable spheres did not require additional maintenance after initial deployment in the field. However, some of these spheres needed to be replaced because rodents and deer frequently ate them. As Stelinski et al. (2001) stated, prevention of deer feeding and inhibition of mold growth are needed before these spheres can be recommended to growers.

The plastic spheres used in 2000 needed successive dipping in pesticide solution to maintain their effectiveness in killing *R. mendax* flies. Depending on the insecticide used, the risks of repeated exposure to the applicator may not justify the use of plastic spheres in this manner. The sucrose caps (Prokopy et al. unpublished) used on plastic and wooden spheres in 2001 may make these spheres more appealing to growers.

Wooden pesticide-treated spheres deployed with a sucrose cap may be another alternative. We noted that the resulting fruit injury from plots treated with wooden imidacloprid-treated spheres was lower than plastic and biodegradable spheres. The major problem with wooden spheres is that they are no longer commercially available; a problem that can be rectified if their usefulness in the cropping system exceeds production costs.

The sucrose cap included in our experiments in 2001 was designed to last for a longer duration in the field compared with earlier versions of sucrose caps. An increase in the duration of available sugar may have lead to an increase in fly kill over time. As Stelinski et al. (2001) noted, pesticide-treated spheres require a constant supply of sugar to act as a feeding stimulant and be effective.

Further research is needed to determine how many spheres are needed to treat different fields possessing varying fly densities. Our results show that plastic spheres may be a viable option to control blueberry maggot. However, there should be a system for releasing a constant supply of sugar such as the sugar caps used in 2001. In addition plastic spheres must maintain the residual efficacy of the pesticide.

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USE OF HOST FRUIT CHEMICAL CUES FOR LABORATORY REARING OF *DORYCTOBRACON AREOLATUS* (HYMENOPTERA: BRACONIDAE), A PARASITOID OF *ANASTREPHA* SPP. (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Doryctobracon areolatus (Szepligeti) (Hymenoptera: Braconidae) is a common parasitoid of *Anastrepha* spp. (Diptera: Tephritidae). An efficient method of laboratory rearing incorporates chemicals from pear fruits into oviposition units. Production for the F_1 and F_2 generations was 12.1 and 9.3 progeny per female, respectively. Mean daily progeny production by F_2 females was between 1-2 progeny per female for almost all ages from 9 to 22 days. A bioassay was designed to determine the source of chemical cues used for host location. Parasitoids were given a choice between two oviposition units: a positive control containing all possible cues, and a treatment unit with cues derived from either the host fly, host fruit, both, or none. The number of females active on each oviposition unit was recorded. This experiment demonstrated that chemical cues derived from the host fruit, probably the peel, are involved in host location.

Key Words: biological control, fruit fly, host location, oviposition

RESUMEN

Doryctobracon areolatus (Szepligeti) (Hymenoptera: Braconidae) es un parasitoide común de *Anastrepha* spp. (Diptera: Tephritidae). Un método eficiente de criarlos en el laboratorio incorpora unos químicos de la fruta de la pera en las unidades de oviposición. La producción en las generaciones F_1 y F_2 fueron 12.1 y 9.3 descendientes por hembra, respectivamente. El promedio de la producción diaria de los descendientes para las hembras de F_2 fué entre 1-2 descendientes por hembra para casi todas las edades de 9 a 22 dias. Un bioensayo fué diseñado para determinar la fuente de las señales químicas usadas para la ubicación del hospedero. Los parasitoides podian escoger entre dos unidades de oviposición: un control positivo que tenia todas las señales posibles, y una unidad de tratamiento con las señales derivadas ya sea de la mosca hospedera, de la fruta hospedera, ó ambas, ó ninguna de las dos. Se registró el número de hembras activas sobre cada unidad de oviposición. Este experimento demostró que las señales químicas derivadas de la fruta hospedera, probablemente la cascara, estan envueltas en la localización del hospedero.

Doryctobracon areolatus (Szepligeti) (Hymenoptera: Braconidae) is a widespread Neotropical parasitoid of *Anastrepha* Schiner spp. (Diptera: Tephritidae), ranging from Mexico to Argentina (Wharton & Marsh 1978). In Brazil, it is the dominant species, constituting between 62% and 89% of all *Anastrepha* parasitoids in various surveys (Canal et al. 1994, 1995; Leonel et al. 1995; Araujo et al. 1996; Aguiar-Menezes & Menezes 1997; Aguiar-Menezes et al. 2001). Furthermore, *D. areolatus* represented 43-59% of all parasitoids collected in the State of Veracruz, Mexico (Hernandez-Ortiz et al. 1994; López et al. 1999), and accounted for 33% of the parasitism in Venezuela (Katiyar et al. 1995).

Doryctobracon areolatus was introduced into Florida in 1969 for the control of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Baranowski & Swanson 1970). It is currently the dominant parasitoid in the interior region of south-central Florida. In a recent study we found that it parasitized up to 36% of the host larvae and constituted 61-100% of all parasitoids at various sites (unpublished data).

Due to the importance of *D. areolatus* as a parasitoid of *Anastrepha* spp., there is much interest in establishing laboratory cultures of this species. Rearing of several fruit fly parasitoids has been facilitated by the use of 'oviposition units' in which host larvae are presented to the females

within an artificial apparatus (Wong & Ramadan 1992). This is based on the finding that female *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) exhibit an ovipositional response to vibrations of the host larvae (Lawrence 1981). However, *D. areolatus* females show no response to hosts in such an apparatus, and rearing has been successful only through the presentation of fruit fly larvae within host fruit.

Several studies have demonstrated the importance of fruit-associated chemicals in host location by parasitoids of fruit flies. Greany et al. (1977) found that chemicals released by fungi associated with rotten fruits are attractive to *D. longicaudata* females. Messing & Jang (1992), using chopped ripe fruits placed in traps, demonstrated attraction of *D. longicaudata* females to various host fruits. Messing et al. (1996) demonstrated similar responses by *Psyttalia fletcheri* (Silvestri) (Hymenoptera: Braconidae) to odors of fresh cucumber and decaying pumpkin.

Parasitoids may also respond to cues associated with the host fly. Prokopy & Webster (1978) found that *Utetes canaliculatus* (=*Opius lectus*) (Gahan) (Hymenoptera: Braconidae) responds primarily to the host-marking pheromone of *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). Similarly, *Halticoptera rosae* Burks (Hymenoptera: Pteromalidae) was found to respond to the pheromone deposited by *Rhagoletis basiola* (Osten Sacken) (Diptera: Tephritidae) (Roitberg & Lalonde 1991).

In this paper we describe an efficient method of *D. areolatus* rearing by incorporating host chemicals into oviposition units. We demonstrate that the ovipositional response is to chemicals derived from the host fruit, and not the fly.

Materials and Methods

Laboratory Rearing

Insects. A parent generation of *D. areolatus*, a total of 128 females and 41 males, was reared from cattley guava, *Psidium cattleianum* Sabine, fruit collected mostly at LaBelle, Florida. Larvae of *A. suspensa* were obtained from a laboratory colony maintained for approximately 150 generations at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida.

Cage Setup. Adult parasitoids were maintained in 20 cm^3 metal-framed cages, the top and two side panels with 16-mesh (per inch) screens, and other panels Plexiglas. One of the side Plexiglas panels included a cloth sleeve. A brown paper towel was taped to the outside of the opposing Plexiglas panel, in order to reduce light intensity. Each cage was stocked over a period of several days (depending on the emergence rate) with up to 100 females and 100 males. Food was supplied daily in the form of a fresh block of honey agar set on an inverted 30 ml plastic cup, and a strip of honey on the Plexiglas side panel. Water was supplied in a 100 ml plastic cup with a cloth wick inserted through a hole in the lid; the external part of the wick was split in half and laid upon the lid. Cages were maintained at 25 ± 0.5°C, 45% R.H., and a light-dark cycle of 14:10.

Oviposition Unit. Oviposition units were composed of *A. suspensa* larvae in diet (Burns 1995) between two layers of cloth, topped with a layer of parafilm, all maintained within a 7.6 cm diameter plastic embroidery hoop. Before exposure to the parasitoids, the parafilm had been wrapped overnight on a fresh 'Anjou' pear (chosen because pears were commercially available throughout the year), previously placed for several hours in a cage with adult *A. suspensa*. The parafilm was placed in the unit with the side previously in contact with the host fruit facing out. This procedure, allowing transfer of fruit chemicals to the oviposition unit, was previously used by Papaj & Prokopy (1986) for fruit fly bioassays. Each sheet of parafilm was used on two consecutive days and, when not in use, was kept in a sealed and refrigerated plastic cup.

Approximately 40 cm^3 diet containing several hundred host larvae were placed in each oviposition unit. The larvae-diet mixture was selected from areas of the larval trays containing high densities of larvae, so that at least 50% of the volume was larvae. This was done to increase the chance of successful probing by the parasitoids. A greater amount of diet would have allowed larvae to migrate away from the oviposition surface and avoid parasitism. Less diet would have left parts of the unit devoid of hosts, thus decreasing the chance of a successful probing. Host larvae were usually 4 or 5 days old, corresponding to late second and/or early third instar; occasionally 3 or 6 day old larvae were used.

The oviposition unit was elevated onto an inverted 100 ml plastic cup to set it closer to the center of the cage, thus improving access of the parasitoids to it. Hosts were exposed to parasitoids for approximately 8 h daily. However, when high activity (15 or more parasitoids simultaneously on the oviposition unit) was observed, two successive exposures were performed, with units being replaced after 4 h. This was done to reduce the chance of superparasitism.

Parasitoids were first provided with hosts within several days of emergence. Exposure continued daily, depending on availability of suitable hosts, until the last female in the cage died. Because cages were stocked over several days, the exact age of ovipositing females could not be determined. Age was estimated as the difference between the exposure date and the median emergence date of all females in a particular cage. This age estimate for F_2 females was subsequently related with the number and sex ratio of their progeny.

Immature Stages and Adult Emergence. Upon completion of exposure, host larvae were transferred to 30 ml plastic cups, which were filled to the top with fresh diet. These cups were then placed upon moist fine vermiculite (15-20 ml water per 100 cm^3 vermiculite) in 500 ml plastic cups. Fully developed larvae emerged from the diet, dropping to the vermiculite in which they pupated. After allowing larvae to pupate for several days, the vermiculite was sieved, and host puparia transferred into fresh moist vermiculite within 100 ml plastic cups. These cups were covered with a solid lid, which was replaced after one week with a cloth lid. This procedure allowed the vermiculite to remain moist while minimizing development of fungi. Immature stages were maintained at the same environmental conditions as adults.

Number and sex of adult parasitoids were determined upon emergence, and adults were transferred to screened cages. Cups were discarded when no emergence was observed for several days.

Bioassay of Chemical Cues

Doryctobracon areolatus were reared successfully from host larvae in oviposition units with parafilm that had contained possible chemical cues from both adult fruit flies and host fruit (described above). A subsequent study was conducted to confirm that chemicals from the host fruit and/or adult fly were used as cues for host location and to further determine the source of these cues.

Insects. Adult *D. areolatus* used in the bioassay were F_3 individuals from the laboratory culture described above. Larvae of *A. suspensa* were obtained from the laboratory culture at the Division of Plant Industry described above.

Experimental Design. Cages were 30 cm long × 20 cm wide × 20 cm high. The bottom and two longer sides were Plexiglas, with a cloth sleeve in the middle of one of the side panels. The top panel was 52-mesh (per inch) screen, and the two smaller sides 16-mesh screen. Each of 6 cages was stocked with 100 female and 70 male *D. areolatus*. Dead females were replaced daily. Before experimentation, females were provided at least once with an oviposition unit containing both host fruit and fly chemicals. Oviposition units were as described above for the laboratory culture, except that the embroidery hoops were made of wood and not plastic.

Parasitoids in each cage were allowed to choose between two oviposition units, both placed upon inverted plastic containers. One unit ('Positive control') contained parafilm wrapped overnight on unwaxed 'Anjou' pears exposed to ovipositing *A. suspensa* females for several hours. This unit contained all possible chemical cues deriving from the host fruit and adult host fly, similar to units used in rearing the laboratory culture.

The second oviposition unit ('Treatment') contained parafilm with chemical cues from either the host fruit or fly, a combination of both, or without added cues. This treatment unit presumably represented a subset of the positive control unit, and response was expected to be either equal to or less than response to the positive control. Treatments were: (1) Untreated parafilm; (2) 'Intact fruit'—wrapped on fresh undamaged pear; (3) 'Punctured fruit'—wrapped on pear punctured approximately 200 times with a no. 0 insect pin (to simulate puncturing by ovipositing flies); (4) 'Damaged fruit'—wrapped on pear from which sections of pulp had been cut out (to simulate vertebrate damage); (5) 'Fly cues'—placed for several hours within a cage containing ovipositing *A. suspensa* females (flies oviposited through the parafilm from several to several hundred times); (6) 'Fly cues + punctured fruit'—as treatment (5) but subsequently wrapped on punctured pear.

The experiment was replicated on 12 of 13 consecutive days. On each day, each of the 6 cages contained a different treatment. Each treatment was replicated twice in each cage, placed alternately on the left and right side of the cage; the placement on any given day was random.

Response Variable and Statistical Analysis. The number of females active on each oviposition unit was recorded at 1, 4 and 8 h following placement of the units in the cage. An active female was defined as an individual either probing into the unit with its ovipositor, or one standing on the unit with ovipositor at a horizontal or below horizontal position; when the female is not reproductively active the ovipositor is curved slightly upward.

The difference between the 'Positive control' and 'Treatment' units ('diff') was calculated for each cage at each hour. This variable was submitted to the MIXED procedure of the SAS statistical software package (Verbeke & Molenberghs 1997), with the hourly observations treated as repeated measurements. This procedure produced t-statistics for each treatment, testing whether the variable 'diff' was different than zero, i.e., whether there was a significant difference between 'Positive control' and 'Treatment'. It further produced t-values comparing 'diff' among the various treatments.

Results

Laboratory Rearing

Lifetime progeny production averaged 2.4, 12.1 and 9.3 for P_1, F_1 and F_2 females, respectively. Mean daily production by surviving F_2 females was between 1-2 progeny per female for almost all ages from 9 to 22 days (Fig. 1).

The sex ratio was 44.7, 62.5 and 48% males for the progeny of P_1, F_1 and F_2 females, respectively. The sex ratio of the progeny of F_2 females was relatively stable over time, averaging close to 50%

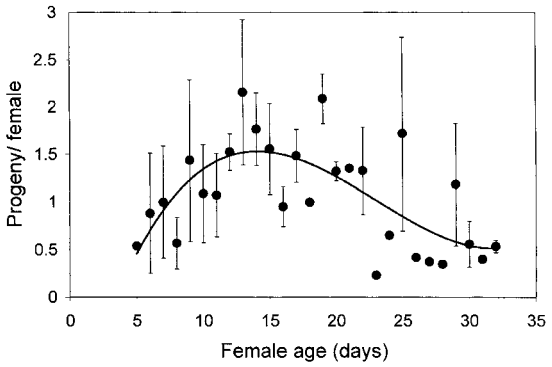

Fig. 1. Daily progeny production by F_2 *Doryctobracon areolatus* females.

(Fig. 2). However, at the oldest female ages the progeny sex ratio tended to be male-biased. This may be the result of sperm depletion, or perhaps lower mortality of unmated females.

Development time of immature stages at 25°C was 22.1 ± 1.1 days (range 19-35 days) for females and 20.6 ± 1.1 days (range 18-26 days) for males.

Bioassay of Chemical Cues

Figure 3 compares the number of active *D. areolatus* females on the oviposition unit among the various treatments. Ovipositional response to the intact and punctured fruit treatments was highest, and did not differ from the response to the positive control ($t = 1.77$, $p = 0.08$; $t = 0.26$, $p = 0.80$; respectively). Response to the fly cues + punctured fruit, damaged fruit, fly cues, and untreated parafilm treatments was significantly lower than to the positive control ($t = 2.24$, $p = 0.03$; $t = 3.83$, $p = 0.0003$; $t = 7.28$, $p < 0.0001$; $t = 6.80$, $p < 0.0001$; respectively).

Adult parasitoid response was greater to all fruit treatments, i.e., intact fruit, punctured fruit, damaged fruit and fly cues + punctured fruit, than to either fly cues only ($t = 3.90$, $p = 0.0003$; $t = 4.96$, $p < 0.0001$; $t = 2.44$, $p = 0.02$; $t = 3.56$, $p = 0.0008$; respectively) or to untreated parafilm ($t = 3.56$, $p = 0.0008$; $t = 4.63$, $p < 0.0001$; $t = 2.10$, $p = 0.04$; $t = 3.23$, $p = 0.002$; respectively). Additionally, response to punctured fruit odor was greater than that to odor of damaged fruit ($t = 2.53$, $p = 0.01$). All other comparisons among treatments were statistically insignificant.

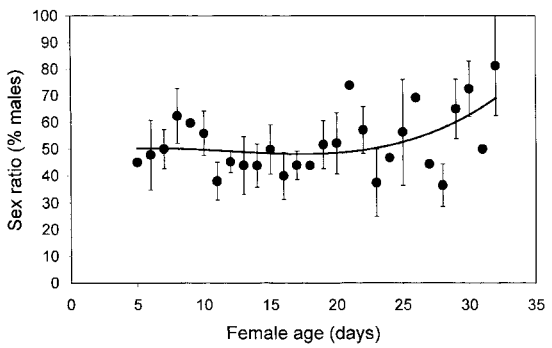

Fig. 2. Relationship between age of F_2 *Doryctobracon areolatus* females and sex ratio of progeny produced.

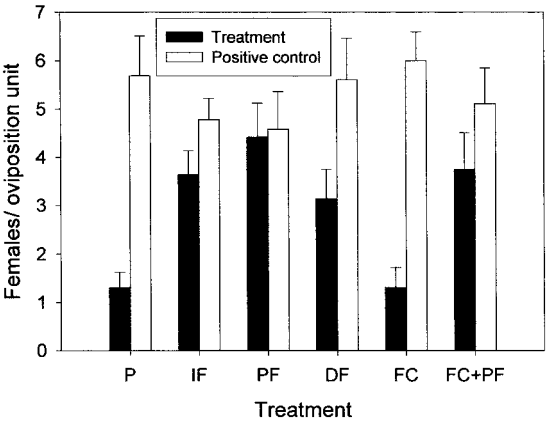

Fig. 3. Number of *Doryctobracon areolatus* females on oviposition units treated with chemical cues from various sources ('Treatment') and on units containing cues from fruit exposed to flies ('Positive control'). The mean value for three observations (1, 4 and 8 h following placement of the units in the cage) is presented. P = Untreated parafilm; IF = Intact fruit; PF = Punctured fruit; DF = Damaged fruit; FC = Fly cues.

Discussion

Parasitoids utilize a wide range of host-related stimuli for host location, often chemical (Godfray 1994). We found that chemical cues emanating from ripe host fruit elicit a significant ovipositional response in *D. areolatus*. Response to "damaged" fruit, in which pieces of peel are removed and the pulp exposed, is somewhat less than to the whole fruit, suggesting that the active chemical(s) may be located in the peel.

Chemical cues derived from the host fly have no apparent effect on *D. areolatus* females. *Utetes canaliculatus* and *H. rosae*, which were shown to respond to host fly pheromones (Prokopy & Webster 1978; Roitberg & Lalonde 1991), parasitize eggs or early-instar larvae, whereas *D. areolatus* prefers later instars. As the host pheromone is water-soluble, it would be degraded by precipitation. Species like *D. areolatus*, which attack the host larvae long after they were deposited as eggs, have less of an association with the pheromone, and thus are less likely evolve a response to it.

Vibration cues are insufficient to elicit a significant ovipositional response in *D. areolatus*. Vibrotaxis has been reported for *Diachasmimorpha*

mellea (Gahan) (Lathrop & Newton 1933), *Diachasma alloeum* (Muesebeck) (Glas & Vet 1983), and *Aganaspis pelleranoi* (Brethes) (Hymenoptera: Eucoilidae) (Ovruski 1994), and *D. longicaudata* can locate larvae within fruit solely by vibration sensing (Lawrence 1981). The lack of response to larvae alone does not imply that host vibrations have no role in host location in *D. areolatus*. Chemical cues may be used in the early stages of host location, as attractants or arrestants, with vibration stimulating probing behavior once the parasitoid is on the fruit.

Total progeny production for *D. areolatus* in the current study was less than half the 29.6 progeny per female reported for *D. longicaudata* (Greany et al. 1976). Similarly, daily progeny production for *D. longicaudata* peaked at nearly 4 progeny per female (Greany et al. 1976), which is approximately double the peak daily progeny production for *D. areolatus*. For both species, the number of mature eggs in the ovaries (*D. areolatus*, 64.3 ± 4.3, n = 6; *D. longicaudata*, 73.0 ± 6.3, n = 6; t = 1.18, p = 0.26; 7-day-old females not exposed to hosts, specimens reared in Mexico by M.A.) is much greater than the maximum number oviposited per day. Thus, the differences in progeny production between the two species are not due to differential egg supply. These may be the result of different experimental procedures or differential adaptability to laboratory conditions. However, they may also represent different reproductive strategies, whereby *D. longicaudata* produces large numbers of progeny in a short period of time, and *D. areolatus* smaller numbers over longer periods.

The rearing method reported here is an improvement over the procedure of rearing *D. areolatus* on host larvae within fruits, and could serve as a basis for the establishment of laboratory cultures for research. Such research could supply further information on life history traits, temperature tolerances, host location, competitive abilities, etc., of *D. areolatus*, which in turn could help explain field observations, e.g., differences in temporal and spatial distribution patterns between *D. areolatus* and *D. longicaudata* in Florida (Sivinski et al. 1998; Eitam unpublished data).

Further improvements in rearing techniques could make possible mass-production for purposes such as augmentative releases (see Sivinski et al. 1996, for an example of augmentative releases of *D. longicaudata*). For instance, chemical identification of fruit cues used for host location may totally eliminate the need for fruits in laboratory rearing.

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NATURAL HISTORY OF *ANISOTA PEIGLERI* (LEPIDOPTERA: SATURNIIDAE) IN GAINESVILLE, FLORIDA

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Populations of a yellowstriped oakworm[1], *Anisota peigleri* Riotte 1975 (Lepidoptera: Saturniidae), occurred at outbreak levels in Gainesville, Florida from 1996 until 2000. The outbreak covered ~1000 hectares centered near Norton Elementary School in the northwest portion of the city. Although not usually an economically important pest, *A. peigleri* invaded homes during mass larval dispersal, defoliated oak trees, and produced large amounts of frass.

This short-lived, localized outbreak in our community gave us the opportunity to document the flight, oviposition, and feeding biology of this little known insect at the southern extreme of its range. We provide this information for those interested in understanding and/or managing this insect.

Anisota peigleri, a recently described species (Riotte 1975), is similar to and was previously identified as *A. senatoria* (J. E. Smith), the orange-striped oakworm. Current knowledge of the natural history, ecology and behavior of *A. peigleri* is summarized in Tuskes et al. (1996). This insect ranges from Kentucky and North Carolina southward to northern Florida; it tends to be common in northeastern Georgia, northwestern South Carolina, and western North Carolina (Riotte & Peigler 1981). During 2002, larvae were collected in Birmingham, Alabama.[2]

Females are reddish-orange in color and have filiform antennae. Their forewings are 24-30 mm long and marked with a small white spot, a dark line, and numerous dark speckles; hindwings are somewhat paler and have an indistinct line. Males are smaller than females and have plumose antennae. The forewings are 18-21 mm long, dark reddish brown with a small white spot and a larger whitish translucent patch. Male hindwings are mostly reddish brown.

During the Gainesville outbreak, adults of *A. peigleri* were found resting on shrubs, tree trunks, and the walls of buildings during the day. Eggs were generally located on the underside of leaves at the ends of the lower branches. Females were occasionally found resting on the base of the leaf after the eggs were laid. Eggs were yellow to orange-yellow, spherical, and about 1 mm in diameter. Following hatch, neonates fed gregariously, skeletonizing small clusters of leaves. Early instars were yellow with a black head capsule and two prominent horns arising from the second thoracic segment. Body coloration changed from yellow to black during the third and fourth instars. Final instars were black with lateral yellow stripes and up to 50 mm long. The two black horns arise prominently from the second thoracic segment and a row of small spines runs the length of the body behind each horn. Mature larvae dispersed from host trees and burrow into the soil to pupate. Pupae reside in the soil about 7-10 cm deep (Felt 1905), remaining there for nearly ten months. An illustrated life cycle can be seen at http://eny3541.ifas.ufl.edu/oakworm/anisota_peigleri.htm.

Adults of this univoltine insect appeared in the late summer. Using field observations and several funnel traps baited with virgin females in each of the three outbreak areas, adults were observed from August 17 to September 22, 1999 & 2000. Peak flight occurred in early September with the greatest count of 20 moths (18 adults field observed and 2 males trapped) being on September 6, 1999.

Female moths tended to lay their eggs on the underside of one leaf on branches closest to the ground. On water oak (*Quercus nigra* L.), egg clusters averaged 154.7 eggs (SE ± 16.7, n = 33) with a range of 10-374. Egg clusters on Shumard oak (*Q. shumardii* Buckl.) averaged 142.6 ± 8.4 eggs with a range of 13-179 (n = 53). Dissections of 29 females yielded abdominal egg counts of 265.8 ± 17.3 with a range of 145-402 eggs. Comparing these fecundity values with the number of eggs on leaves suggests that females normally deposit all their eggs in one cluster. Small egg clusters are likely a result of disturbance during oviposition.

Egg development times were monitored in the lab in environmental chambers adjusted daily to mimic outdoor conditions (temperature and photoperiod). Oviposition to hatch ranged from 6 to 10 days and averaged 9.1 days (n = 1757 eggs). Egg viability was high (>95%) but egg masses were regularly parasitized by *Anastatus reduvii* Howard (Hymenoptera: Eupelmidae). On average 30% of eggs in an affected egg mass were parasitized. Egg clusters deposited early in the flight period suffered less parasitism (~30% of eggs) than those deposited later in the flight season (~50% of eggs).

[1]We used the name yellowstriped oakworm to have a descriptive name while working with the general public. Larvae have a distinct yellow lateral stripe that is easily seen and recognizable. This name is also to the liking of Richard S. Peigler (pers. comm.), for whom the insect was named.

[2]Mature larvae collected from *Quercus falcata* Michx. on 9 August 2002 in Birmingham, Shelby Co., Alabama were shipped to us for identification. Larval characters described by Riotti and Peigler (1981) confirmed they were *A. peigleri*. Voucher specimens are in the Florida State Collection of Arthropods, Gainesville, Florida.

The most common hosts of *A. peigleri* in Gainesville were water oak and Shumard oak. Southern red oak (*Q. falcata* Michx.) and laurel oak (*Q. laurifolia* Michx.) were infested less frequently. We observed ultimate instars browsing and occasionally feeding on live oak (*Q. virginiana* Mill.) but, because early instars did not survive on it, we do not consider live oak to be a host.

In laboratory studies of larval survival and development times, we used 3 replicates of 120 larvae on cut foliage from each of 3 hosts. Percent survival from hatch to pupation averaged 14.2% ± 1.5 on Shumard oak and 13.2% ± 1.4 on water oak. No larvae survived into the second instar on live oak, suggesting its unsuitability as a host. For larval development times there was no significant difference (t-test, p = 0.607) between larvae reared on water oak and those reared on Shumard oak. Pooling all tests on the two hosts, larval development at 24°C averaged 48 days with instars 1-5 lasting 12, 8, 8, 9 and 11 days respectively.

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Summary

The yellowstriped oakworm, *Anisota peigleri*, occurred at outbreak levels from 1996 until 2000 in Gainesville, Florida. This population was in the southern most extreme of this species' range. We documented various aspects of its natural history and biology including host trees, egg mass characteristics, egg development, larval development and survival.

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REDISCOVERY OF *LIGYROCORIS SLOSSONI* (HEMIPTERA: LYGAEOIDEA: RHYPAROCHROMIDAE), A RARELY COLLECTED SEED BUG CONSIDERED PRECINCTIVE IN FLORIDA

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Since its original description nearly 90 years ago, *Ligyrocoris slossoni* Barber has remained a rarely collected lygaeoid bug whose habits are unknown. Only the unique holotype and three additional specimens have been recorded (Sweet 1986; Slater & Baranowski 1990), and information on its habitat is limited to Blatchley's (1926) comment that he collected a female at Dunedin, Florida, "by beating dead leaves of oak near the bay beach."

Barber (1914) described *L. slossoni* from a male taken at Lake Worth, Florida, but in his revision of *Ligyrocoris*, he omitted *slossoni* from his keys, noting that his description of this now "doubtful species" had been based on a damaged and apparently teneral specimen (Barber 1921). When a fully sclerotized specimen became available for study (the female from Dunedin), Barber (1924) was able to reinstate *L. slossoni* as a valid species and to redescribe it.

Barber subsequently identified this seed bug from other southeastern states and from as far north as southern Illinois (Sweet 1986). In a list of the Lygaeidae of Iowa and Illinois, Slater (1952) recorded it from Washington County, Illinois, based on Barber's determination. Sweet (1986), however, found that material identified as *L. slossoni* actually represented two species, with nearly all specimens proving to be an undescribed species that he named *L. barberi*. Sweet (1986) also reported two additional specimens of true *L. slossoni*; both were from Alachua County, Florida, with one labeled as taken at Gainesville.

The Illinois record of *L. slossoni* (Slater 1952), although retained (as *L. slossonae*; see discussion of nomenclature below) in the most recent catalog of North American lygaeoids (Ashlock & A. Slater 1988), evidently is based on a misidentification. Sweet's (1986) type series of *L. barberi* included a specimen from Dubois [Washington Co.], Illinois, which likely is the one on which the Illinois record of *L. slossoni* had been based.

Both *L. barberi* and *L. slossoni* have a transverse dark fascia on the corium (Sweet 1986). *Ligyrocoris slossoni* can be distinguished by the characters given by Sweet (1986) in his key to eastern species of the genus and by his illustrations of the head, pronotum, hemelytra, abdomen, and fore femur. Characters that facilitate recognition of *L. slossoni* include the head unicolorous dark red (dark brown in *L. barberi*), pronotum with only a few scattered hairs (densely hairy in *L. barberi*), pronotal collar with distinct groove (collar not set off by distinct groove in *L. barberi*), metapleuron shiny (pruinose in *L. barberi*), femora and tibiae reddish and contrasting with the yellow tarsi (legs pale yellow, except distal 2/3 of femora light reddish brown, in *L. barberi*), and fore femur with one major spine (two in *L. barberi*) (Sweet 1986).

On the basis of recent field work in Florida, I here provide additional records of this rarely collected rhyparochromid (see Henry [1997] for current classification of lygaeoid families) and notes on its habits and the habitats in which it was taken. Voucher specimens have been deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C.

In his original description of *L. slossoni*, Barber (1914) stated that the holotype was from the collection of Mrs. Annie T. Slosson, implying (but not explicitly stating) that he was naming the species for her. Assuming that Barber intended to dedicate the species to her, his latinization was incorrect. Barber's name, however, cannot be considered an incorrect original spelling, and the emendation *L. slossonae* (Ashlock & A. Slater 1988) should be regarded as an incorrect subsequent spelling under Article 32.5 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). It might be argued that the use of *L. slossonae* by Slater and Baranowski (1990), the only literature reference to this species subsequent to Ashlock and A. Slater's (1988) emendation, represents prevailing usage under Article 33.3.1 of the Code. Because of Article 32.5 of the Code and lack of an explicit statement by Barber (1914) regarding use of the name *slossoni*, I am retaining Barber's original spelling.

Material examined (all collections by the author; roman numerals = nymphal instars): FLORIDA: Hamilton Co., jct. Rt. 129 & SW 79 Terrace, 0.3 km N of Suwannee River, 2 km NE of Suwannee, 30°23.8'N, 82°56.0'W, 1♀, ex crown of *Andropogon tenuispatheus* (Nash) Nash (Poaceae), 27 May 2000; Lake Co., Rt. 27, 8.5 km S of jct. Rt. 50, SSE of Clermont, 28°28.7'N, 81°43.0'W, 21♂, 14♀, 1 III, 2 IV, 4 V, ex crowns of *Eragrostis curvula* (Schrad.) Nees (Poaceae), and 4♂, 1♀, 1 V, ex basal rosettes of *Heterotheca subaxillaris* (Lamarck) Britton & Rusby = *H. latifolia* Buckl. (Asteraceae), 20-22 Mar. 2002; 2 V, ex crowns of *E. curvula*, 3 Nov. 2002; 2 ♂, 1♀, 2 III, 4 IV, 3 V, ex crowns of *E. curvula*, 21 Nov. 2002.

Of the 115 lygaeoid species recorded from Florida, 10 are known only from that state (Baranowski 1995) and are considered precinctive—that is, confined to that area (see Frank & McCoy 1990). *Ligyrocoris slossoni* is one of those lygaeoids known only from Florida (Slater & Baranowski 1990), yet its recent collection in Hamilton County, which borders on Georgia, suggests that it also might be found in southern Georgia. Its distribution, though, apparently is more restricted than that of the widespread and morphologically similar *L. barberi* (Sweet 1986).

I collected *L. slossoni* in the eastern panhandle of Florida (Hamilton Co.) in a ruderal site along Rt. 129 in an area of mesic flatwoods (see Wheeler [2001] for more information on the habitat). Collections in Lake County also were made in a field-type habitat (*sensu* Sweet 1964) along Rt. 27 and about 10 meters inside a fence on adjoining property of Lake Louisa State Park. This disturbed site near the northern end of the Lake Wales Ridge has scattered weeping lovegrass plants, prickly pear (*Opuntia humifusa* [Raf.] Raf.; Cactaceae), plus camphorweed (*H. subaxillaris*), ragweed (*Ambrosia artemisiifolia* L.; Asteraceae), and other rank forbs. Myodochine rhyparochromids beaten from crowns of grasses with *L. slossoni* at both sites were *Paromius longulus* (Dallas) and *Perigenes similis* Barber. The presence of *L. slossoni* in ruderal habitats contrasts with those of *L. barberi* in Texas: prairies rather than roadsides and early successional stages (Sweet 1986).

My experience with *L. slossoni* in Hamilton County is consistent with that of all previous collectors—that is, only a single adult was found. At this site on 2-3 June and 29 November 2000, I was unable to collect additional adults from crowns of broomsedge or to sweep adults from several species of Asteraceae.

In southeastern Lake County, I collected 40 adults in late March 2002; 35 were beaten from crowns of weeping lovegrass, mainly from dead plants or those with some dead stems. At the same time, seven mid- to late instars of presumably *L. slossoni* were beaten from lovegrass crowns and one fifth instar was beaten from vegetative growth of camphorweed. In further observations at this site on 13 April 2002, 10 adults, three late instars, and an apparent cast skin of *L. slossoni* were beaten from weeping lovegrass; 10 adults, two late instars, and a cast skin were beaten from camphorweed. The adults and nymphs observed on 13 April were replaced on plants and are not listed above under material examined. My sampling of camphorweed and weeping lovegrass at the Lake County site on 2 June and 10 August 2002 did not yield additional nymphs or adults of *L. slossoni*. I did not sample the litter layer where this seed bug, like *L. diffusus* Uhler and many other ground-inhabiting rhyparochromids (Sweet 1964), might feed more on fallen seeds than on the seed heads of plants. At this same site in early and late November 2002, third through fifth instars and adults again were found in crowns of weeping lovegrass.

The mid- to late instars and adults collected in late March and observed in mid-April in Lake County likely are those of a first generation. It is not known if *L. slossoni* overwinters as diapausing eggs, as does *L. barberi* in Texas (Sweet 1986). If eggs of *L. slossoni* overwinter, then the adult collected by Blatchley at Dunedin in mid-February also would be that of a first generation. The nymphs and adults found in November likely are those of at least a second generation. Populations of *L. slossoni* might be only bivoltine, as are those of *L. barberi* in Texas despite the long, warm season (Sweet 1986).

It is unknown if nymphs of *L. slossoni* develop mainly on a particular plant species. Sweet (1986) determined that *L. barberi* feeds on ripe seeds of the composite *Rudbeckia hirta* L. and that the bug's seasonality closely parallels host phenology. Even though nymphs and an apparent exuviae of *L. slossoni* were beaten from lovegrass, nymphs are unlikely to complete development on this plant. During the day, adults and nymphs might use crowns of weeping lovegrass for shelter or to conserve water under xeric conditions and at night forage in the litter layer for seeds of composites. Nymphs might also feed on seeds of other plants that lodge or accumulate in the extensive crowns of this African bunchgrass (see Wheeler 1999). The collection of smaller numbers of adults, a late instar, and a cast skin from *H. subaxillaris* suggests an association with this composite, but its role in the bug's population dynamics remains unknown. Nymphs fed on ripe seeds of *H. subaxillaris* in the laboratory.

The collections of *L. slossoni* reported here appear to be the first for this species since at least the 1940s. Only one of the four previously known specimens bears a label giving year of collection (1923, Alachua Co.). In addition to the holotype (Barber 1914), the adult that Blatchley (1926) collected in February at Dunedin must have been taken between 1913 and 1926 (see Blatchley 1930). The specimen from Gainesville labeled "JRW 5413" likely was collected by J. R. Watson. He joined the Department of Entomology at the University of Florida, Gainesville, in 1911, was a thysanopterist and avid collector of other insect groups, and died in 1946 (Tissot 1946).

The rediscovery of this myodochine rhyparochromid in Florida, though increasing the number of known museum specimens from four to 51 (including four reared from fifth instars), does not explain its rarity in collections. Is this seed bug restricted to certain habitats because of competition with other rhyparochromid seed predators? Is its life cycle closely associated with seed pro-

duction of one or a few host plants? Data that might help answer these and other questions about its bionomics must await field and laboratory studies comparable to those on *L. barberi* in Texas (Sweet 1986) or on *L. diffusus* and other rhyparochromids in Connecticut (Sweet 1964).

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Summary

Described in 1914 and apparently not collected since at least the 1940s, *Ligyrocoris slossoni* was found recently at two sites in Florida. In Lake County, 38 adults and 18 mid- to late instars were beaten from crowns of weeping lovegrass (*Eragrostis curvula*; Poaceae) in March and November 2002; at the same site, five adults and a fifth instar were taken on camphorweed (*Heterotheca subaxillaris*; Asteraceae). One adult was beaten from the crown of a broomsedge (*Andropogon tenuispatheus*; Poaceae) in Hamilton County. These collections increase the number of known museum specimens from four to 51 (including four reared from fifth instars).

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TOXICITY OF *BACILLUS THURINGIENSIS* CRY1-TYPE INSECTICIDAL TOXIN TO GEOGRAPHICALLY DISTANT POPULATIONS OF TOMATO PINWORM

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The tomato pinworm (TPW), *Keiferia lycopersicella* (Walsingham), is an important pest of tomato in southern California (Oatman 1970), Texas (Wellik et al. 1979) and Florida (Poe 1974). In Florida this insect pest increases to tremendous numbers late in the tomato season (namely in spring) when the plants mature. In a number of instances, dense populations (2 to 10 larvae/leaf) have resulted in large scale death of the plants, despite frequent insecticide applications (D.R.S, field observation).

Currently growers use chemical insecticides to manage TPW in commercial fields; but the degree of control is not satisfactory in large measure because of the deficiencies in the current spray programs and detection method. The use of broad-spectrum insecticides for TPW control may induce development of resistance, or induce outbreaks of secondary pests (Smith 1970; Brown & Pal 1971). Cultural practices such as burning of crop residues, crop rotations, manipulation of planting dates, etc. helped suppress the TPW population (Elmore & Howard 1943; Poe 1974). However, insecticides are the only tool for pest management that is reliable for emergency action when insect pest populations approach or exceed the economic threshold (Metcalf 1975). Therefore, efforts are needed to evaluate less detrimental insecticides for control of TPW.

Bacillus thuringiensis has become an effective insecticide in the management of various insect pests (Burgerjon & Martouret 1971); however, resistance to *B. thuringiensis* toxin has been documented through laboratory studies in 12 species of Lepidoptera, 2 species of Coleoptera and 5 species of Diptera (Tabashnik 1994). When compared to the response of a fully susceptible strain the level of resistance observed in most instances did not exceed 50- to 100-fold.

For coping with resistance to *B. thuringiensis* protein toxins, it will be very important to determine the level of resistance that a given population can develop, and the cross-resistance spectrum of this resistance. Considerably more information of benefit to management would be obtained if the mode of inheritance of the resistance could also be determined. The overall objective of the present study was to characterize the toxicity of a selected *B. thuringiensis* toxin to a number of geographically diverse populations of the TPW.

Insects. The study was conducted in a private quarantine facility at Labelle, Florida. Mixed instars of TPW were collected from tomato over a period of time from 4 locations in Florida (Tropical Research and Education Center, Homestead; commercial field, Homestead; BHN Laboratories, Naples; commercial field, Naples); three commercial locations in California (Cameron, Hurron, California-South) and three commercial locations in Sinaloa, Mexico (2 locations at Guasava on two dates, and LaPalma). TPW larvae from each location were shipped to Labelle in strict accordance with quarantine procedures. All shipments were secured in the quarantine facility to separate the live TPW larvae. A colony from each geographic population was established on 'Flora-Dade' tomato transplants within the quarantine facility in a separate room to prevent any intermixing. The escape of live insects was prevented by not handling any insects outside the quarantine facility. Each colony was maintained for 2 generations prior to use in the bioassay study. Sufficient numbers of the required developmental stage of TPW were collected from the cultures to run bioassays using the selected *B. thuringiensis* toxin. At the end of each bioassay all live specimens were destroyed by heating in an autoclave.

Bacillus thuringiensis toxins. Sufficient amount of selected toxin of *B. thuringiensis* var. *kurstaki* (Cry 1, Monsanto) was supplied by BHN Research, Bonita Springs, Florida. The toxin was stored in a refrigerator at 6°C at Tropical Research and Education Center, Homestead, Florida for future use.

Bioassay procedure. A leaf-dip bioassay was conducted in the laboratory. Five concentrations of the selected *B. thuringiensis* toxin (0, 6.25, 12.50, 25.00, 50.00 and 100.00 µg/ml of water) were prepared in 0.02% Tween 20 (Sigma St. Louis, MO). The concentrations of *B. thuringiensis* protein were prepared following a serial dilution method, where a factor of 0.5 was used in each dilution step. Each concentration was made up to a total volume of 20 ml in a test tube. Freshly cut tomato leaflets (eight leaflets/concentration) were immersed in each suspension for one minute. The leaves were removed and air dried. To avoid leaf desiccation, the petiole of each leaf was wrapped with moist cotton, which was kept moist by adding a drop of distilled water daily.

Two treated leaflets from each concentration were placed in a petri dish (9 cm. diam.) and infested with 8 freshly molted 2nd/3rd instars (4 larvae/leaflet). Bioassays were repeated four times. Bioassays were maintained at 28 +1.2°C, 75-81% r.h., & 14 h photoperiod in the quarantine facility. All concentrations of *B. thuringiensis* toxin were tested at one time against a specific population and placed on a bench in a randomized complete block design.

Evaluation of experimental treatments was made by recording mortality of the TPW larvae at 24-h intervals for 4 days after initiation of the experiment. Any larva that failed to move when touched repeatedly was considered dead.

Statistical analysis. The LC_{50} values and confidence limits were obtained by probit analysis (POLO-PC, LeOra Software 1987). In the present study, nontreated control mortality was below 20%, hence Abbott's formula (Abbott 1925) was not used to correct TPW mortality data.

LC_{50}s among the different populations were similar with a greatest difference of 3.17-fold. The slope values generated from dose-mortality response varied significantly among the TPW populations. The slope value of TREC population is almost 3-5 times greater than other experimental populations (Table 1). This high slope value is not unusual for a laboratory culture reared for several generations, resulting in a more homogeneous population. The lower slope values for the field population are typical, indicating greater heterogeneity. Hemingway et al. (1993) concluded heterogeneity in resistant strains of German cockroaches based on the shallow slopes of the probit lines.

The LC_{50} value for the TREC population was similar to that of the LaPalma population, but the confidence interval for the LC_{50} of the LaPalma population was wider than that of the TREC population (Table 1). The LC_{50} values of the TREC and LaPalma populations did not differ from the commercial field population at Homestead and the BHN laboratory population at Naples. The LC_{50} values of the rest of the experimental populations were higher than populations of Homestead (laboratory and field), BHN and LaPalma. With the increase of LC_{50} values in other populations, confidence intervals increased. The confidence interval reflects the extent of variability in the response of individuals of a population to a certain concentration.

In contrast to the LC_{50} value, the LC_{90} value of LaPalma population was 3 times greater than the TREC population (Table 1). With the TREC colony, the highest concentration tested was at least 3 times higher, and significantly different based on the upper limit of 95% confidence limit (CL), than the concentration needed to cause 90% mortality in the same population (Brewer et al. 1990; Brewer & Trumble 1991; Sanderson & Roush 1992). With the rest of the experimental populations, the concentrations of toxins needed to cause 90% mortality were 3 to 30 times more than the highest concentration tested.

Field to susceptible ratios (FS) were >1.0 for most of the populations when 50% of a population was considered to cause mortality (Table 1). FS (LC_{90}) values were 2 to 4 times greater than the corresponding FS (LC_{50}) values.

Based on the present study, TPW is susceptible to the Cry1-A endotoxin of *B. thuringiensis*. The concentrations needed to cause adequate mortality of TPW must be determined based on the variability of susceptibility in widely separated geographical populations.

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TABLE 1. SUSCEPTIBILITY OF TOMATO PINWORMS, *KEIFERIA LYCOPERSICELLA*, FROM VARIOUS GEOGRAPHICAL AREAS TO INSECTICIDAL CRYSTAL PROTEIN FROM *BACILLUS THURINGIENSIS* VAR. *KURSTAKI*.

Population*	Slope + SE	LC_{50} ($FL_{95\%}$[2])	LC_{90} ($FL_{95\%}$)	FS[1] (LC_{50})	FS (LC_{90})
1. Lab., Homestead	9.97 ± 2.26[3]	17.68 (14.88-21.00)	23.77 (20.15-32.44)	—	—
2. Field, Homestead	2.61 ± 0.54	22.74 (16.88-31.33)	70.59 (46.38-170.89)	1.28	2.96
3. BHN Lab.	1.75 + 0.37	27.15 (18.34-45.05)	146.94 (107.73-1449.83)	1.53	6.18
4. Field, Naples	1.69 + 0.40	44.45 (29.38-83.68)	255.39 (119.00-1782.65)	2.51	10.74
5. Cameron	2.20 + 0.46	32.59 (22.92-47.75)	124.51 (75.38-366.56)	1.84	5.23
6. Huron	1.72 + 0.41	42.54 (28.00-72.87)	237.12 (117.18-1428.03)	2.40	9.97
7. LaPalma	1.94 + 0.49	17.79 (12.58-30.79)	81.58 (41.66-534.43)	1.01	3.43
8. Guasava 1	1.72 + 0.44	56.00 (36.83-116.93)	311.16 (138.68-3047.21)	3.17	13.01
9. Guasava 2	3.32 + 0.39	42.18 (18.97-833.68)	102.62 (39.54-32504.63)	2.39	4.31

*1: TREC, Homestead laboratory colony; 2: commercial field, Homestead, FL; 3: BHN Laboratory, Naples, FL; 4: commercial field, Naples, FL; 5: Cameron, CA; 6: Huron, CA; 7: LaPalma, Sinaloa, Mexico; 8: Guasava 1, Sinaloa, Mexico; 9: Guasava 2, Sinaloa, Mexico.

[1]FS (Field to susceptible ratio: lethal concentration (LC) value of the field population divided by the lethal concentration value of the reference susceptible strain.

[2]95% CI (confidence interval) were calculated from probit analysis.

[3]Data are expressed in micrograms of toxin per ml of water. Number of replications for each population: 4.

critical comments and suggestions on the early draft of this manuscript.

Summary

The susceptibilities of nine geographically distant populations of tomato pinworm, *Keiferia lycopersicella* (Walsingham), to Cry1-A protein produced by *Bacillus thuringiensis* var. *kurstaki* are presented. LC_{50} values were similar with a difference of 3-fold. The slope values for different populations varied significantly, indicating a variability in the susceptibility among the populations to the Cry1-A. This information will establish a basis for selecting a proper concentration of *B. thuringiensis* toxin to be used for the control of this economically important pest on tomato genetically engineered for resistance to this pest. The results also provide baseline data for monitoring resistance.

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MATING PROPENSITIES FROM DIFFERENT RATIOS OF MALE AND FEMALE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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The sterile insect technique (SIT) is used as a preventative measure against the establishment of wild populations of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in California and Florida. It involves the mass-release of sterile flies that mate with wild flies, when present, resulting in nonviable progeny. The replacement of bisexual strains with male-only, genetic sexing strains [e.g., strains carrying a temperature sensitive lethal (*tsl*) mutation] has occurred, or is anticipated, in many sterile release programs, because of their increased effectiveness in suppressing pest populations (Rendon et al. 2000). The release of far fewer females (<1%) than males results in a male-biased sex ratio that could change field mating dynamics. Medflies exhibit a lek mating system, in which males congregate to attract potential mates, and female choice determines male success (Emlen & Oring 1977; Prokopy & Hendrichs 1979; Whittier & Kaneshiro 1995; Eberhard 2000). In a field study in Hawaii, Shelly et al. (1994) found an average of 4 wild males/lek. Our research examined different ratios of male and female flies to determine their effects on the amount of time until, and probability of, mating pair formation.

Male Mediterranean fruit flies used in mating tests were from the Vienna-7/Tol-99 (males-only, *tsl* genetic sexing) strain and were obtained as puparia from the California Department of Food and Agriculture laboratory in Waimanalo, HI. Flies were irradiated 2 d before eclosion at 145 Gy, at the USDA laboratory in Waimanalo. Males were provided with water and sugar. Female flies used in mating tests were obtained from T. Shelly's laboratory (USDA-APHIS, Manoa, HI), and were from a stock of flies in the 6th generation from wild flies collected as larvae from Jerusalem cherry (*Solanum pseudocapsicum* L.). Virgin females, isolated from males less than 24 h after eclosion, were provided water and fed honey, sugar, and protein hydrolysate until they were sexually mature.

Male flies used in mating tests were 6-9 d-old and females were 7-14 d-old, both sexually mature. Tests occurred between 0900 and 1200 in transparent, upside-down, plastic containers (266 ml) that were isolated from each other using paper dividers (18 cm × 25 cm). A total of 80 replicates were completed (March 2002), with one replicate consisting of 5 containers each with a different combination of males (m) and females (f): 1m, 1f; 1m, 2f; 1m, 3f; 2m, 1f; and 3m, 1f. Males were aspirated into containers 10-15 min prior to females. Containers were checked every 3-5 min for mating pairs and when found, the time from female introduction was recorded. Observations were made until 90 min after female introduction.

Logistic regression was used to analyze the proportion of males and females mating and the amount of time prior to mating pair formation was analyzed using an ANOVA with means separated by Fisher's LSD test (SAS Institute 1999).

There were significant fly sex effects on the proportion of flies mating (Likelihood ratio 19.97; df 2; χ^2(prob) < 0.001). Female ratio effects were significant (χ^2 = 17.54, df 1, χ^2(prob) < 0.001), while male ratio effects were not (χ^2 = 3.06, df 1, χ^2(prob) < 0.08). Equal sex ratios resulted in the lowest number of mating pairs, while higher ratios of females to males resulted in a larger proportion of flies mating than comparable ratios of males to females (Fig. 1). There were no overall ratio effects on the time to form a mating pair (F = 2.14; df 4, 273; P = 0.076), but data suggest that skewed ratios (1:2, 1:3, 2:1, and 3:1) resulted in mating pairs forming earlier than for a 1:1 ratio (Fig. 2).

The sequence of male behaviors leading up to copulation are 1) pheromone calling by males, 2) orientation to the female (after the female has arrived), 3) continuous wing vibration, and 4) intermittent wing buzzing/head rocking (Eberhard 2000). Routine inspection of containers indicated that almost all males exhibited precopulatory be-

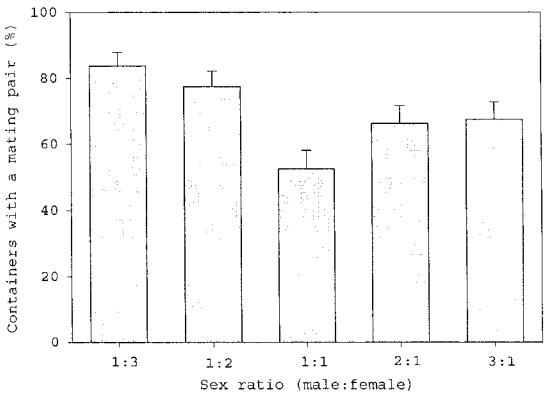

Fig. 1. Frequency of Mating Pair Formation. Percent of containers (mean ± SE) with a mating pair for different sex ratios of Vienna-7 males and 6th generation wild females. (Likelihood ratio 19.97, df 2, χ^2(prob) < 0.001; female ratio effects: χ^2 = 17.54, df 1, χ^2(prob) < 0.001; and male ratio effects: χ^2 = 3.06, df 1, χ^2(prob) < 0.08).

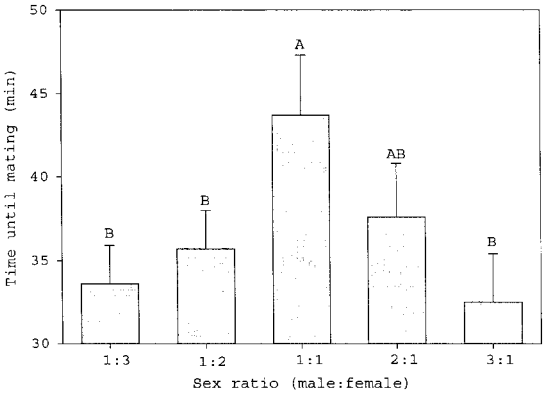

Fig. 2. Time Until Mating Pair Formation (min). Time to form a mating pair (mean ± SE) for different sex ratios of Vienna-7 males and 6th generation wild females. (ANOVA $F = 2.14$; df 4, 273; $P = 0.076$). Means were separated using Fisher's LSD test. Containers with no mating pairs were excluded from the analysis.

havior (i.e., steps 1-4 above). Interpretation of results is in reference to females, because Mediterranean fruit flies use a lek mating system, where females control choice of a mate (Arita & Kaneshiro 1985). When one female was in a container with two or three males, females could assess mate potential by discriminating among several males. In containers with one female and one male, females were less likely, and took longer to mate. This suggests that mate selection by females was slowed and inhibited because of an absence of rival males that could be compared. When two or three females were in a container with only one male, females were likely less selective because of competition with other females, and mated sooner. An alternate explanation is that female acceptance thresholds were constant but variable among individuals and the likelihood of including a female with relatively low acceptance criteria increased with an increasing number of females.

These mating trials were unusual in that they allowed evaluation of precise sex ratios, because in each treatment, one sex was limited to a single individual (or both sexes in the case of the 1:1 ratio). This resulted in a constant sex ratio for each container. In typical experiments with unequal sex ratios, the operational sex ratio changes as mating pairs form (i.e., without fly replacement to keep the sex ratio constant). In addition, sex ratios can vary locally within a field cage. Shortcomings of our study that make it of less direct value to field-based SIT programs include (1) experiments were performed in the laboratory and (2) because of the experimental design, different fly densities were present with different treatments.

Although male-biased ratios should make females more selective for mates, within the protocols of current SIT release programs, females would have a large supply of sterile males from which to choose.

Summary

Increasing the total number of flies in a laboratory container to form skewed ratios of high males or females, resulted in a higher proportion of mating pairs and their formation earlier. There were significant female ratio effects on the proportion of flies forming mating pairs. Male ratio effects were not statistically significant but suggested a trend similar to female effects.

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