LARVAL AGGREGATION AFFECTS FEEDING RATE IN CHLOSYNE POECILE (LEPIDOPTERA: NYMPHALIDAE)

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ABSTRACT

Larvae of *Chlosyne poecile* (Felder) (Lepidoptera: Nymphalidae: Melitaeini) on *Razisea* sp. (Acanthaceae) feed in large aggregations as early instars but disperse and feed in small groups or as solitary caterpillars in later instars. The effect of group size on per capita feeding rate was tested by manipulating the number of larvae on a leaf and measuring the leaf area eaten in short-term feeding trials. Feeding rate increased significantly with group size for first instars but decreased with group size for all larger instars. Although feeding rate decreased significantly with group size for second instars, second instars in the field were usually found in large groups and did not begin to disperse until the third instar. Variance-to-mean ratios indicate that larval aggregation was lower in later instars, slowly approaching a random distribution. The distributions of larger instars may therefore be the result of random dispersal to food and not the active avoidance of other larvae. If the observed lag between the predicted optimal time to disperse and the observed pattern is adaptive, then it may be due to unmeasured benefits of aggregation, such as lower predation rates and unmeasured costs of dispersal. Egg clustering and aggregation of larvae may be more common for butterflies in the Neotropics than in other areas.

Key Words: caterpillars, feeding facilitation, group defense, Costa Rica, Neotropical Lepidoptera

RESUMEN

Las larvas de *Chlosyne poecile* (Lepidoptera: Nymphalidae) comen hojas se *Razisea* sp. (Acanthaceae). Durante sus primeros estadios se formen agregados grandes, pero se dispersan y alimentan en grupos pequeños o solitariamente en los ultimos estadios. El efecto del tamaño de las agregaciones en las tasas de alimentación per capita fue puesto a prueba manipulando el número de larvas por hoja y metiendo el área de la hoja que a sido comida. La tasa de alimentación aumentó significativamente junto con el numero de individuos presentes en la hoja para el primer estadio, pero disminuyó en relación al tamaño del grupo para todo los demás estadios. Aunque la tasa de alimentación disminuyó significativamente con tamaño del grupo para las larvas del segundo estadio, estas larvas usualmente se encuentaron en grandes grupos en el campo, y su dispersión ocurrió solo en el tercer estadio. Las tazas promedio de varaza indicante que la agregación larval fue menor en los últimos estadios, aproximándose lentamente a una distribución tipo Poisson. Esto sugiere que las distribuciones de los estadios más grandes son el resultado de la dispersión al azar hacia el alimento y no un meanismo activo para evitar a otras larvas. Se propone que el retraso en la predicción del tiempo óptimo para la dispersión y el patron observado es debido de beneficio obtenidos al agregarse que no he sido cuantificado, como una reducción en las tasas de depredación y costos de dispersión. Las agregaciones de larvas y huevos de mariposas pueden ser mas frecuentes en el Neotropico que en otras áreas.

Translation provided by Authors.

Although most Lepidoptera lay single eggs and develop as solitary larvae, 5% to 15% of butterfly species are reported to lay eggs in large clusters and have larvae that feed gregariously during early instars (Stamp 1980). This life history has arisen independently in many different lineages but, it is especially common among the Nymphalidae and is common for species in the genus *Chlosyne* (e.g., Scott 1986; DeVries 1987; Clark & Faeth 1997; Denno & Benrey 1997). Proposed selective advantages of larval aggregation include increased feeding efficiency (Clark & Faeth 1997; Denno & Benrey 1997), enhanced group defense against predators (Stamp 1980; Chew & Robbins 1984; Vulinec 1990), and improved thermoregulation (Tsubaki 1981; Stamp & Bowers 1990; Casey 1993; Bryant et al. 2000). Selective pressures may lead females to oviposit in large clutches (Stamp 1980; Courtney 1984; Heard & Remer 1997), causing at least the first instars to be aggregated by default until they disperse to feed individually.

Aggregation may increase feeding efficiency by allowing early instars to overcome leaf toughness by a "group attack" in one spot (Ghent 1960), by

overwhelming plant defenses (Storer et al. 1997), or by laying down an architecture of silk that aids the larvae in feeding (Rathcke & Poole 1975; Fitzgerald 1995). Higher larval feeding rate can reduce development time, potentially both raising intrinsic rate of increase and decreasing the duration of larval exposure to parasitoids and abiotic mortality sources (Clancy & Price 1987; Benrey & Denno 1997). Higher larval feeding rates also can result in larger adults, a characteristic that is positively correlated with fecundity in many insects (e.g., Juliano 1998).

Aggregating caterpillars often exhibit aposematic coloration and distastefulness (Sillén-Tullberg & Leimar 1988; Vulinec, 1990). Aggregation may therefore enhance the effects of caterpillar defenses because predators learn to avoid larvae after eating a few distasteful individuals (Sillén-Tullberg & Leimar 1988). Alternatively, the advantage of aggregation may be not to the larvae but to the ovipositing female or the eggs. Fresh females in the genus *Chlosyne* are often so heavy with eggs that they can barely fly (DeVries 1987), so the female may need to unload the eggs quickly because of energetic costs of carrying so much weight in flight or higher predation risk due to reduced evasion ability. Moreover, "dumping" eggs quickly may be advantageous to the female, especially when adult mortality risk is high, even if it lowers the average fecundity of the offspring (Courtney 1984). Egg clusters may also suffer a lower intensity of parasitoid attack than solitary eggs (Morrison & Strong 1981).

Many species with gregarious larvae, and most if not all in the genus *Chlosyne*, only feed in large groups as early instars, becoming increasingly solitary through later instars (Clark & Faeth 1997; Benrey & Denno 1997). Several hypotheses, which are not mutually exclusive, can explain this across-instar decrease in aggregation. If larvae are initially aggregated for increased feeding efficiency, these benefits may disappear in later instars as the larvae increase in size and are able to penetrate the leaf cuticle and/or overwhelm plant defenses without assistance. Moreover, effects of competition for leaf area may be greater in larger larvae and thus depress the feeding efficiency of groups. Group advantages for defense against predators may diminish in later instars if larger larvae are exposed to fewer potential predators or if later instars have more effective defense mechanisms (Stamp 1986). For example, later instars may have sequestered defensive compounds that were unavailable earlier, the aposematic coloration of individuals may be more visible, or larger larvae may deliver a more potent dosage of defensive compounds. If larvae were initially aggregated only because of advantages to the ovipositing female or the eggs, then larvae could be less aggregated as later instars merely because of random dispersal patterns.

Our objective was to characterize the effect of group size on the short-term larval feeding rate for different instars of *Chlosyne poecile* (Felder). This species is locally abundant in northwestern Costa Rica, where it feeds on shrubs in the family Acanthaceae in second-growth habitats and in light gaps. We hypothesize that any benefit to feeding in larger groups will be largest for the first instar and decrease for later instars, perhaps even becoming a cost of group feeding in the later instars. Finally, a review of the natural history of Costa Rican Lepidoptera suggests that aggregative behaviors (egg clustering and gregarious feeding) are more common in the Neotropics than in other regions of the world. Whether the difference is caused by differences in selective pressures among the regions deserves further research.

METHODS

Study System

Chlosyne poecile is found from Costa Rica to Venezuela (DeVries 1987). In Costa Rica it is locally abundant from sea level to 900 m on the Pacific slope in dry forest and semideciduous forest. Casual observations by the authors in multiple years suggest that *C. poecile* is abundant during the rainy season and rare or absent in the dry season, a pattern typical for this genus in the Neotropics (DeVries 1987). DeVries (1987) reports the eggs, larval stages, and host plants for *C. poecile* as unknown. We observed females ovipositing on, and larvae of all instars feeding on, a woody shrub in the family Acanthaceae, which we identified to the genus *Razisea* on the basis of vegetative characters. All egg clusters we found were on the undersides of leaves near the top of the plant. Newly laid eggs were yellow and turned tan and then brown shortly before hatching on their fifth day.

The study was conducted in the forest immediately adjacent to the Estación Biológica San Miguel (EBSM) within Cabo Blanco National Park, Puntarenas Province, Costa Rica. The maritime forest around EBSM is mostly 35-year-old second growth (C. Castrillo, pers. comm.). *Razisea* sp. grows commonly in the understory near EBSM, especially along trails and at the edges of gaps. Caterpillars of *C. poecile* were extremely abundant on the *Razisea* sp. shrubs growing near the station. *Razisea* sp. and *C. poecile* caterpillars also were found less abundantly at the edges of light gaps along streams near EBSM at 50–100 m elevation. All of the plants and larvae in the censuses and experiments were located along the "beach trail" at the EBSM. The experiments described below were initiated during the Organization for Tropical Studies course 2000-3 and were conducted from 16 to 22 July 2000.

Larval Group Size and Aggregation

We used two types of censuses to quantify larval aggregation. In the first census we searched for *C. poecil*e larvae and recorded the instar and number of larvae in a group. These data were used to estimate the average group-size for each instar and revealed the instar at which larvae switched to feeding individually. In the second census we searched every leaf of 14 *Razisea* sp. plants and recorded the number of empty leaves as well as the group size and instar for all larvae we encountered. We used these data to estimate the degree of aggregation of larvae for each instar by calculating the variance-to-mean ratio of the number of larvae per leaf, including unoccupied leaves on the same plant. The degree of aggregation of the larvae was compared with the expected variance-to-mean ratio (equal to one) for a random (Poisson) distribution with a chi-square test (Krebs 1999).

Effects of Group Size and Instar on Feeding Rate

We estimated larval feeding rates for different instars by placing an individual or a group of sibling larvae on the underside of a single, large, undamaged leaf and measuring the leaf area eaten within a given amount of time. Leaves are lanceolate and approximately 15-25 cm long and 7 – 10 cm wide, and larvae were corralled on a leaf by a band of Tanglefoot Tangle-Trap® smeared around the petiole. No larvae were observed trying to cross the band of Tanglefoot®. Larvae were left to feed for 4 to 25 h, and all replicates of the same instar started and stopped at approximately the same time. Smaller instars were left to feed longer than larger instars. The leaf area eaten was estimated with gridded transparencies. Results are expressed as the leaf area eaten (in square millimeters) per hour per larva or per larval volume, based on the average volume measured for 10 individuals of each instar. Average volumes were estimated to be 0.72 mm^3 for first, 4.56 mm^3 for second, and 36.82 mm^3 for third instars and 149.92 mm^3 for the fourth and fifth instars, which differed more in head capsule size and coloration than in estimated volume. The results were analyzed by single classification ANOVA and regressions. By using as wide a range of group sizes as possible, instead of replicating only a few group sizes, we were better able to characterize the effects of group size and look for nonlinear effects of group size, including an intermediate optimum. All analyses were done with S-Plus 6.1 (Insightful Corporation 2001).

The *C. poecile* larvae fed both day and night, and on 12 leaves the groups of larvae ate too much of the leaf to permit accurate estimation of the leaf area removed. These leaves were excluded from further analyses, leaving 19 groups of first instars, 19 groups of second instars, 9 groups of third instars, and 16 groups of fourth/fifth instars. Group sizes in the final data ranged from 1 to 100 larvae, but instars differed in the maximum number of larvae in a group, because fewer late instars would fit on a leaf. Fourth and fifth instars were lumped together to increase the sample size of these late instars. The largest group of fourth and fifth instars that we used was 10 caterpillars, which consumed most of a large leaf in a few hours.

Results

Larval Aggregation

The mean group size for egg masses and each instar counted in the first census are shown in Fig. 1. The variance-to-mean ratio calculated from the second census was high in the second instars, intermediate for first and third instars, and near one for fourth and fifth instars. First instars were consistently highly aggregated in large groups. The larvae began to disperse in the second instar and continued in the third, so some of these larvae were found in large groups whereas others were found as solitary larvae, thus inflating the variance-to-mean ratio. Fourth and fifth instars were mostly found as solitary caterpillars and data suggest their distributions were not significantly different from a random distribution among all possible leaves, although sample sizes for these instars were too low to warrant a formal test.

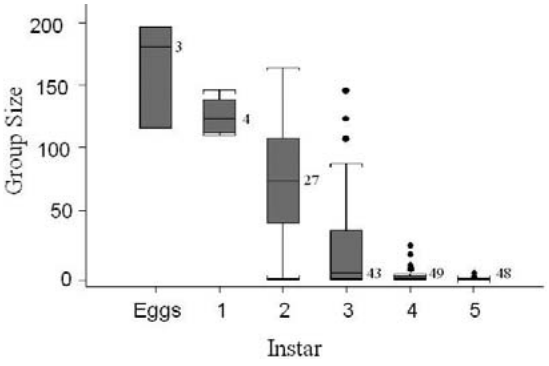

Fig. 1. Group sizes observed for eggs and for instars 1-5. The box contains 50% of the data, and the median is indicated by a line. The whiskers contain approximately 95% of the data, and outliers beyond the whiskers are shown as points. Observed group size decreased with increasing instar. The sample size for each instar is shown beside its box.

Effects of Group Size and Instar on Feeding Rate

Instars differed tremendously in feeding rate. Larger larvae ate more leaf area per hour than small larvae ($F_{1,59} = 58.227$, $P < 0.0001$), but when feeding rate was expressed as leaf area consumed per hour per unit larval volume, the effect of instar on feeding rate was no longer significant ($F_{1,59} = 2.087$, $P = 0.154$). Feeding rate increased significantly with group size for the first instars (Table 1, Fig. 2). Nine of the 12 groups of first instars with fewer than 25 individuals never began feeding, whereas all 7 larger groups of first instars fed. Solitary first instars either never attempted to feed or were unable to break the leaf cuticle. The feeding rates decreased with group size for the second, third, and fourth/fifth instars, but this decrease was statistically significant only for the second instars (Table 1, Fig. 2). The effect of group size was therefore smaller in later instars. For all instars the effects of group size were linear; no higher-order terms were significant. Because the much smaller range of group sizes tested for the fourth and fifth instars would obscure interactions involving group size, we used data only for first, second, and third instars in a single-classification ANOVA. The interaction between group size and instar was significant ($F_{1,43} = 4.526$, $P = 0.039$); that is, these smaller instars differ significantly in the slopes of the relationships between feeding rate and group size.

Discussion

Our results clearly support the hypothesis that larval group size positively affects *C. poecile* feeding rate for first instars. First instars construct a sparse network of silk that appears to help them feed, and they appear less likely to deposit silk and begin feeding successfully when in small groups. Surprisingly, larval aggregation becomes a disadvantage for the second instars of *C. poecile*. These results are contrary to those of Denno & Benrey (1997), who found a positive effect of group size on larval growth rate in second instars of a congener, *C. janais* (Drury). The maximum group size that these authors tested was less than half of the mean group size in the field, however, so they would not have detected effects that became apparent only with larger feeding groups. Clark & Faeth (1997) concluded that feeding facilitation at least partly explained shorter development time from hatching to third instar in larger groups of *C. lacinia* larvae, but they did not separate group-size effects among instars. Our study supports their finding of feeding facilitation, but also shows large across-instar differences in feeding rate, suggesting different benefits and costs for different instars.

Larval aggregation and average group size decreased with increasing instar number, approaching a random distribution among available leaves by fourth instars. This behavior is consistent with the lack of significant effect of group size on feeding rate in the third through fifth instars. If intraspecific competition reduced the feeding rate of larger instars in groups, then we would expect the larvae to become overdispersed by avoiding other larvae. Instead, later instars seem to take random walks to search for available leaves and thus only slowly approach a random distribution among available leaves. In contrast, when the first and second instars disperse, they follow silk trails from their old leaves to new leaves, as do those of *C. lacinia* Geyer (Bush 1969), retaining most of their original feeding groups. Dispersal from aggregations in the congener *C. janais* occurs when the caterpillars reach a certain body length, often in the middle of an instar rather than at an instar transition (Denno & Benrey 1997). A similar pattern in *C. poecile* could explain the high level of variation observed in second and third instar group sizes.

Our results clearly indicate that on the basis of feeding rate alone, larvae should disperse as soon as they molt into the second instar. Why do many groups remain aggregated through the second and into the third instar when earlier group dispersal might increase feeding rate? Group thermoregulation is probably not an important factor because larvae were observed feeding through the night in this warm tropical climate. Unmeasured predation, parasitism, or travel costs may drive the delay in larval dispersal. Because early instars move much more slowly than the later instars (personal observation), the costs of travel

Table 1. Summary of linear relationships between feeding rate [leaf area eaten (mm^2) per larva per day per average larva volume (mm^3)] and group size for different instars. The positive effect of group size on feeding rate decreased with increasing instar.

Instar	Slope	$F_{1,(n-1)}$	*P*-value	Sample size *n*
First	2.5224	28.328	<0.0001	19
Second	–1.9494	8.770	0.009	19
Third	–2.6172	3.889	0.089	9
Fourth/fifth	–0.0968	0.002	0.965	16

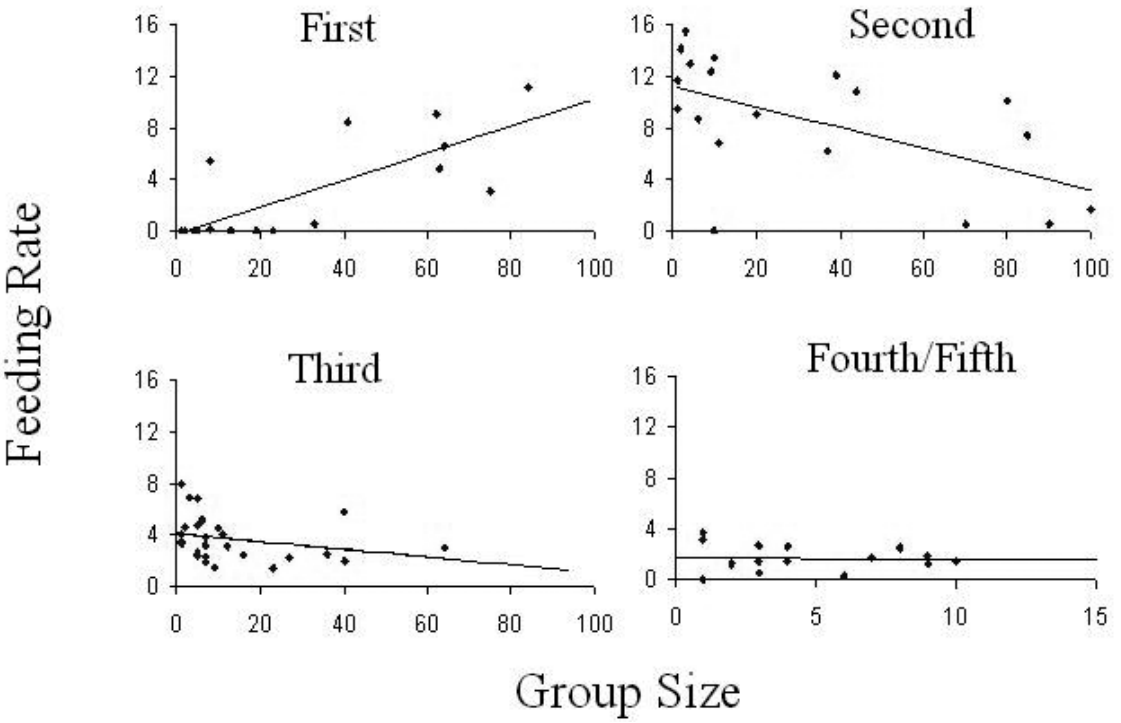

Fig. 2. The relationship between feeding rate [leaf area eaten (mm^2) per larva per hour per average larva volume (mm^3)] and group size (number of individuals) for different instars. The lines are linear regressions. Note that the range of group sizes for fourth/fifth instars is much smaller than that for the other instars. Feeding rate increased significantly with group size for the first instars then decreased significantly with group size for the second instars, and tended to decrease with group size with third and fourth/fifth instars.

time to a new leaf and risk of falling off the host plant may be substantial for even second instars. Moving to new feeding areas may also increase a caterpillar's risk of predation (Bernays 1997).

The changes in appearance of larvae in successive instars suggest that larger caterpillars are also better able to defend against natural enemies. The early instars have few spines and are tan to brown in color. Late instars have typical aposematic coloration; they are black with red heads and occasional orange markings on their backs. The spines on late instars are larger, more numerous, and mildly urticating, causing an itching rash on sensitive skin (personal observation). Although we do not have data on the palatability of the different instars, we noted that the most aposematic larvae fed individually, whereas the least aposematic larvae fed in large groups. Increased feeding efficiency could select for the aggregation of first instars, regardless of their distastefulness, and might be a more important factor than predation for this species. Changes in the risk of parasitism across instars also could contribute to their distributions.

Despite the short duration of the experiments, we were able to find significant effects of group size on the larval feeding efficiency of *C. poecile*. The large feeding groups of first instars and solitary feeding of late instars appear to maximize their feeding rates, but second instars present a conundrum because they were commonly observed in relatively large groups even though their feeding rate declines with group size. We did not examine the costs and benefits of group size for predator defense, which may help to explain the behavior of these caterpillars. We encourage further, more detailed studies of the costs and benefits of insect group-feeding behaviors for different instars, because these costs and benefits are likely to change with instar.

Here we present the first report of gregarious feeding in *C. poecile* larvae, although the behavior was previously known in congeners (DeVries 1987; Clark & Faeth 1997; Denno & Benrey 1997). Egg-laying behavior and gregarious feeding are nearly always coupled. For example, nearly all lepidopterans, including *Chlosyne*, that exhibit gregarious feeding also lay eggs in clusters (and vice versa) in species for which complete data are available—29 out of 30 in Costa Rica (DeVries 1987, 1997; present study) and 22 out of 23 in North America (Stamp 1980). To determine

how common these aggregative behaviors are in Neotropical Lepidoptera, we reviewed the natural history of Costa Rican lepidopterans (DeVries 1987, 1997) and found that, of 234 species for which information is available, 26% lay eggs in clusters. This figure is much higher than estimates for other regions of the world: 5% in North American, 13% in Great Britain, 6% in Australia-New Guinea, and 3% in India (see Stamp 1980 for review). Moreover, although Stamp (1980) found that egg clustering is generally predominant in just one butterfly group per region, families in Costa Rica show consistently high levels of egg clustering: 26% in Papilionidae, 44% in Pieridae, 22% in Nymphalidae, and 32% in Riodinidae. These results raise the question of whether selective pressures acting on Lepidoptera in the Neotropics differ from those in other regions of the world. We suggest further research that compares top-down (predators and parasitoids) and bottom-up (leaf toughness and feeding-induced resistance) effects on gregarious feeding by larvae in the Neotropics with those in other regions of the world.

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REFERENCES CITED

BENREY, B., AND R. F. DENNO. 1997. The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78: 987–999.

BERNAYS, E. A. 1997. Feeding by lepidopteran larvae is dangerous. Ecol. Entomol. 22: 121–123.

BRYANT, S. R., C. D. THOMAS, AND J. S. BALE. 2000. Thermal ecology of gregarious and solitary settle-feeding nymphalid butterfly larvae. Oecologia 122: 1–10.

BUSH, G. L. 1969. Trail laying by larvae of *Chlosyne lacinia*. Ann. Entomol. Soc. America 62: 674–675.

CASEY, T. M. 1993. Effects of temperature on foraging of caterpillars, pp. 5-28 *In* N. E. Stamp and T. M. Casey [eds.], Caterpillars: Ecological and Evolutionary Constraints on Foraging. Chapman and Hall, New York. 587 pp.

CHEW, F. S., AND R. K. ROBBINS. 1984. Egg laying in butterflies, pp. 65-79 *In* R. I. Vane-Wright and P. R. Ackery [eds.], The Biology of Butterflies. Princeton University Press, Princeton, N.J. 429 pp.

CLANCY, K. M., AND P. W. PRICE. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. Ecology 68: 736–738.

CLARK, B. R., AND S. H. FAETH. 1997. The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. Ecol. Entomol. 22: 408–415.

COURTNEY, S. P. 1984. The evolution of egg clustering by butterflies and other insects. American Nat. 123: 276–281.

DENNO, R. F., AND B. BENREY. 1997. Aggregation facilitates larval growth in the Neotropical nymphalid butterfly *Chlosyne janais*. Ecol. Entomol. 22: 133–141.

DEVRIES, P. J. 1987. The Butterflies of Costa Rica and Their Natural History. Volume I: Papilionidae, Pieridae, Nymphalidae. Princeton University Press, Princeton, N.J.

DEVRIES, P. J. 1997. The Butterflies of Costa Rica and Their Natural History. Volume II: Riodinidae. Princeton University Press, Princeton, N.J.

FITZGERALD, T. D. 1995. The Tent Caterpillars. Cornell University Press, Ithaca, N.Y.

GHENT, A. W. 1960. A study of group-feeding behavior of larvae of the jack-pine sawfly *Neodiprion pratti banksianae* Roh. Behaviour 16: 110–148.

HEARD, S. B., AND L. C. REMER. 1997. Clutch-size behavior and coexistence in ephemeral-patch models. American Nat. 150: 744–770.

INSIGHTFUL CORPORATION. 2001. S-Plus 6.1. Insightful, Seattle, Wash.

JULIANO, S. A. 1998. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition. Ecology 79: 255–268.

KREBS, C. J. 1999. Ecological Methodology. Second Edition. Addison-Wesley, Menlo Park, Calif.

MORRISON, G., AND D. R. STRONG. 1981. Spatial variation in egg density and the intensity of parasitism in a Neotropical chrysomelid (*Cephaloleia consanguinea*). Ecol. Entomol. 6: 55–61.

RATHCKE, B. J., AND R. W. POOLE. 1975. Coevolutionary race continues: butterfly larval adaptation to plant trichomes. Science 187: 175–176.

SCOTT, J. A. 1986. The Butterflies of North America. Stanford University Press, Stanford, Calif.

SILLÉN-TULLBERG, B., AND O. LEIMAR. 1988. The evolution of gregariousness in distasteful insects as a defense against predators. American Nat. 132: 723–734.

STAMP, N. E. 1980. Egg deposition in butterflies: why do some species cluster their eggs rather than deposit them singly? American Nat. 115: 367–380.

STAMP, N. E. 1986. Physical constraints of defense and response to invertebrate predators by pipevine caterpillars (*Battus philenor*: Papilionidae). J. Lepid. Soc. 40: 191–205.

STAMP, N. E., AND M. D. BOWERS. 1990. Variation in food quality and temperature constrain foraging of gregarious caterpillars. Ecology 71: 1031–1039.

STORER, A. J., D. WAINHOUSE, AND M. R. SPEIGHT. 1997. The effect of larval aggregation behavior on larval growth of the spruce bark beetle *Dendroctonus micans*. Ecol. Entomol. 22: 109–115.

TSUBAKI. Y. 1981. Some beneficial effects of aggregation in young larvae of *Pryeria sinica* Moore (Lepidoptera: Zygaenidae). Res. Popul. Ecol. 23: 156–167.

VULINEC, K. 1990. Collective security: aggregation by insects as a defense, pp. 251-288 *In* D. L. Evans and J. O. Schmidt [eds.], Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators. State University of New York Press, Albany, N.Y. 482 pp.

ENHANCED OVIPOSITION IN THE INSIDIOUS FLOWER BUG, ORIUS INSIDIOSUS (HEMIPTERA: ANTHOCORIDAE) WITH A PARTIALLY PURIFIED NUTRITIONAL FACTOR FROM PREY EGGS

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ABSTRACT

The insidious flower bug, *Orius insidiosus* (Say), can be maintained on a minimal artificial diet composed of brewers yeast, soy protein hydrolysate and chicken yolk. However, egg production is poor even though the level of protein in the diet exceeds the amount consumed by adults that are fed insect eggs and have higher levels of egg production. We therefore fractionated eggs of the almond moth, *Ephestia kuehniella* Zeller by preparative isoelectric focusing and bioassayed the resultant fractions in test diets. Ovipositional rates were evaluated using a short 1-week bioassay. Adult predators were placed on the diets the third day after eclosion, allowed to feed for six days, and then provided with an oviposition substrate for 24 h on day seven. Egg production significantly increased only in a fraction with an isoelectric point of pH 5. SDS-PAGE revealed the presence of several Commassie blue-stained bands; however, the nature of the factor is unknown. These results point to a fecundity factor required by females of *O. insidiosus* for egg laying that potentially may be used to supplement artificial diets for *Orius* species by commercial producers of beneficial insects.

Key Words *Orius insidiosus*, *Ephestia kuehniella*, predator, artificial diet, oviposition, prey eggs, proteins

RESUMEN

El chinche insidiador de flores, *Orius insidiosus* (Say), puede ser mantenido sobre una dieta artificial mínima compuesta de levadura de cerveza, hidrolisado de proteína de soya e yema de huevo de gallina. Sin embargo, la producción de huevos es pobre a pesar de que el nivel de proteína en la dieta excede la cantidad consumida por los adultos alimentados con huevos de insectos y con un nivel de producción de huevos mas alto. Por eso, nosotros fraccionamos los huevos de la polilla de almendra, *Ephestia kuehniella* Zeller utilizando el enfoque preparativo isoelectrico y por el bioensayo de las fracciones resultantes de las dietas probadas. Las tasas de oviposición fueron evaluadas utilizando un bioensayo corto de una semana. Los adultos depredadores fueron sujetos a las dietas el tercer dia después de eclosionar, se los permitio la alimentación por seis dias y en el septimo dia fueron proveidos con un substrato para la oviposición por 24 h. La producción de huevos aumento significativamente una fracción solamente con un punto isoelectrio de pH 5. La PAGINA-SDS reveló la presencia de varias bandas de 'Commassie' de tinte azul; sin embargo, la naturaleza del factor es desconocida. Estos resultados indican que hay un factor de fecundidad requerido por las hembras de *O. insidiosus* para la oviposición de los huevos que potencialmente puede ser usados para suplementar las dietas artificiales para las especies de *Orius* por los productores comerciales de insectos beneficos.

The insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) is a generalist feeder of thrips, aphids, mites and whiteflies, and eggs of other insects in the field (Barber 1936; McCaffrey & Horsburgh 1986; van der Veire & Degheele 1992; van Lenteren et al. 1997; Funderburk et al. 2000). This predator has been reared in the laboratory on an artificial diet devoid of any insect host components (Weiru & Ren 1989). However, predators reared on this artificial diet had reduced fecundity (Ferkovich & Shapiro 2004a). A general problem associated with a number of other species of predators reared on artificial diets has been a reduction in reproductive rate (De Clercq & Degheele 1992, 1993a & b; De Clercq et al. 1998; Wittmeyer & Coudron 2001). The reason for the reduced fecundity observed in predators fed artificial diets is not clear.

Since adult females of *O. insidiosus*, as with other heteropteran predators such as *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) (Shapiro et al. 2000), exhibited higher yolk content in developing oocytes as well as higher egg production when fed prey versus artificial diet (Shapiro & Ferkovich 2002), we surmised that natural prey may contain a specific nutritional factor needed by the predator for egg production. When adult *O. insidiosus* were fed an artificial

diet, the females exhibited poor egg production even though the level of protein in the diet exceeded the amount consumed per day by adults fed eggs of the Indian meal moth, *Plodia interpunctell*a (Hübner) (Lepidoptera: Pyralidae) When a protein extract from Plodia eggs was tested as a supplement to the *Orius* diet, it significantly increased egg production at concentrations of protein that were 8.3-, 55.7-, and 83.7-times lower than the concentrations needed for beef liver, bovine serum albumin, and chicken egg albumin, respectively (Ferkovich & Shapiro 2004a). Subsequently, Ferkovich & Shapiro (2004b) found that the egg protein extract could be replaced in the diet with cells from an embryonic cell line (PIE) derived from *P. interpunctella* eggs to enhance oviposition of *O. insidiosus*.

In view of some of the positive effects on the rate of oviposition of *O. insidiosus* fed diet containing prey egg-extracted protein (Ferkovich & Shapiro 2004a), we fractionated the proteins in prey eggs to determine if the increased rate of oviposition could be attributed to a specific fraction of proteins.

Materials and Methods

Preparation of Egg Protein Extract

Soluble egg proteins were isolated from 5 g of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (1.25×10^6 eggs) as described by Ferkovich & Shapiro (2004a). Briefly the eggs were homogenized in ammonium acetate buffer (pH 7.5) and the soluble proteins were separated by centrifugation. The soluble proteins were then run through a desalting column, freeze-dried, and stored at -80°C. The freeze-dried desalted powder (352 mg) was then added to 58 ml of distilled water; and the soluble protein concentration of the solution was determined to be 174 mg/total vol.

Protein Assay

The Lowry procedure (Protein Assay Kit, Sigma, St. Louis, MO) was used to assay the quantity of soluble proteins in the egg protein solution and in the fractions after isoelectric focusing.

Preparative Isoelectric Focusing

Five ml of the soluble protein solution and 3 ml of ampholyte solution (pH range 3-10, Bio-Rad, Hercules, CA) were mixed in 42 ml of 1 M urea to prevent loss of proteins due to excessive precipitation. The protein solution was run in a Rotofor Cell© isoelectric focusing unit (Bio-Rad instruction manual) for 2.5 h at 12 W constant power and 4°C. The initial conditions were 408 V and 38 mA and 668 V and 23 mA at equilibrium. Twenty fractions were collected and their volumes (approx. 2.0 ml each) and pH values measured. Fractions 12-17 were cloudy and contained precipitates. Ampholytes were used to form the pH gradient in which the egg proteins were separated. These ampholytes were then removed due to interference with the subsequent protein assay and SDS-gel electrophoresis. They were removed by adding NaCl to each fraction to a final concentration of 1 M for 15 min and dialyzing against water. Aliquots of 10 or 20µl of each fraction were analyzed for protein content. After the fractions were analyzed for protein, they were combined based on the protein profile. Fractions with low protein levels (1-8, 9-12, 18-20) were combined, and ones with higher protein concentrations (13-17) were kept as individual fractions. Each combined or individual fraction was then concentrated to 0.5 ml in a Centriprep© concentrator (10k molecular weight (MW) cutoff; Millipore, Bedford, MA) and 10 or 20µl of each fraction were used to analyze for soluble protein.

Assay of Isoelectric Focusing Fractions in Diet

The 0.5 ml-fractions obtained from the isoelectric focusing of the *Ephestia* egg proteins were each added to 0.5 ml of diet and encapsulated (20µl vol.). Artificial diet was prepared under aseptic conditions in a clean room and encapsulated in stretched Parafilm® with a diet encapsulation apparatus (Analytical Research Systems, Gainesville, FL) described earlier (Carpenter & Greany 1998, Ferkovich et al. 1999). Artificial diet was prepared as described for rearing *O. sauteri* (Weiru & Ren 1989), and consisted of 0.33 g brewers yeast, 0.03 g sucrose, 0.18 g soy protein acid hydrolysate, 3.8 mg of 99% palmitic acid (all from Sigma, St. Louis, MO), 0.04 g chicken egg yolk, and 0.08 g honey in 1.0 ml of distilled water containing the concentrated fractions from isoelectric focusing. Palmitic acid was mixed with the egg yolk component before adding it to the diet.

Newly emerged adults of a Florida strain of *O. insidiosus* (<24 h after eclosion) were obtained from a commercial producer of beneficial insects (Entomos, Gainesville, FL) and placed on the diets on the third day after eclosion. At the end of the sixth day, one 7-cm section of green bean pod, as a substrate for oviposition, was placed in each jar for 24 h. Eggs deposited in the green beans were then counted under a microscope. The insects were held in a growth chamber at 25.5 ± 1°C, with 75 ± 5% RH and a photoperiod of 15:9 (L:D) h. The treatment diets were (1) Eggs (standard)—jars contained 150 *Ephestia* eggs (approx. 3 mg) each as a reference standard; (2) Diet (control)—jars contained artificial diet with no additional substances as control diet; and (3) Diet (amended)—jars contained artificial diet supplemented with each of combined fractions 1-8, 9-12,

18-20, and individual fractions 13 through 17 as separate treatments.

Electrophoresis

Fractions resulting from the separation of the *Ephestia* egg proteins by isoelectric focusing were analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). Gradient SDS-PAGE (4-20%) was carried out in minivertical gels (Bio-Rad) as described by Shapiro et al. (2000).

Data Analysis

Each treatment was replicated four times with six females and four males per replicate. The egg counts were adjusted for female mortality within each treatment. Data were analyzed by ANOVA with StatMost software (Dataxiom Software Inc.). Dunnett's test was used to determine if the number of eggs oviposited per female on each of the diet treatments supplemented with the isoelectric focusing fractions was significantly greater than the number of eggs oviposited per female on the control diet. Since insectaries generally produce *O. insidiosus* on eggs of *E. kuehniella*, we used them as a reference standard but did not include the treatment in the ANOVA.

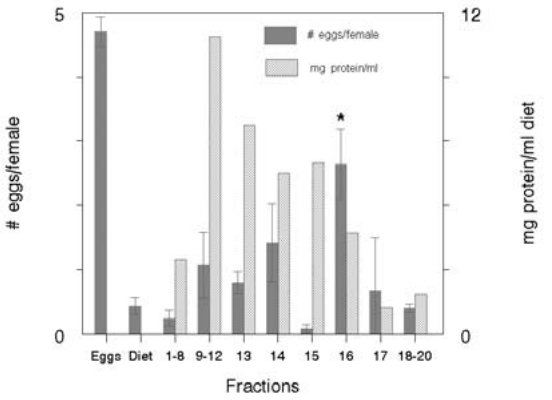

Fig. 2. Average number of eggs (±SE) oviposited by females of *O. insidiosus* after being fed artificial diet supplemented with protein in fractions from isoelectric focusing separation of *Ephestia* egg protein shown in Fig. 1. Eggs (standard)—whole eggs of *E. kuehniella*; Diet (control)—diet with no additional substances; and Diet (amended)—supplemented with each of combined fractions 1-8, 9-12 and 18-20 and individual fractions 13 through 17 in separate diet treatments. Dunnet's test was used to compare the amended treatment diets against the Diet (control); asterisk indicates that the treatment means were significantly different from Diet (control) ($P < 0.05$).

Results

Figure 1 shows the protein profile versus pH of the *Ephestia* egg protein extract separated on in a pH gradient of 3-10. Average rate of eggs oviposited per female was significantly increased relative to the control Diet in only one Diet (amended) treatment, a fraction with an isoelectric point of pH 5 fractions (Fig. 2). The active fraction contained 6.8 mg or 16% of the total protein recovered in all the fractions (43.3 mg). The active fraction (#16) shown in the diet bioassay of the fractions in Fig. 2 contained one major band at 47,000 MW that also appeared to be present as a lighter band in fractions 17 and 18-20 (Fig. 3). Other faint bands at 163k, 51k, 39k, 31k, 27k, and 23k MW were present in fraction 16 (Fig. 3). Recovery of the total protein applied to the gradient was

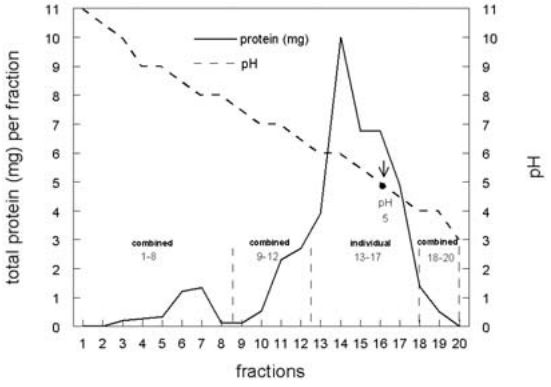

Fig. 1. Protein profile of *Ephestia kuehniella* egg protein separated by isoelectric focusing on a pH gradient of 3-10. Fractions that were combined for bioassay in artificial diet are shown by vertical dotted lines. Arrow indicates the fraction that stimulated the rate of oviposition.

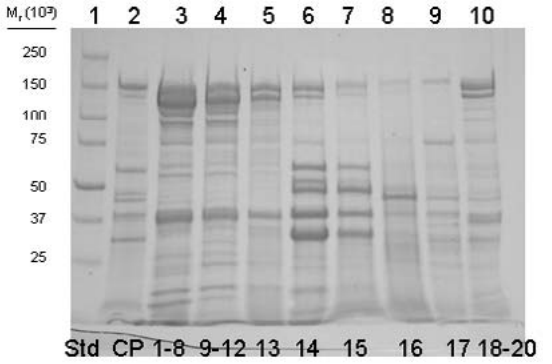

Fig. 3. SDS-PAGE analysis of fractions separated as shown in Fig. 1. Lane 1 (Std) - MW standards; lane 2 (CP)—crude protein; lane 3—combined fractions 1-8; lane 4—combined fractions 9-12; lanes 5-9—individual fractions 13-17; and lane 10—combined fractions 18-20.

24.8% (174 mg applied; 43.2 mg recovered); heavy precipitates in fractions 12-17 after isoelectric focusing resulted in a loss of protein in these fractions when the samples were dialyzed and concentrated.

Discussion

The fecundity of females was increased with the addition of a specific fraction of *Ephestia* egg proteins that were separated by isoelectric focusing. The effect was not dependent on the concentration of egg proteins in the fraction as other fractions that contained higher levels of protein did not stimulate the ovipositional rate of *Orius* females. SDS-PAGE analysis of the fractions revealed major proteins with relative molecular weights ranging between 100k and 150k and less than 40k MW which were not present in the active fraction. Although we did not identify the yolk proteins in *Ephestia kuehniella*, Shirk (1984) identified four major yolk proteins ranging in molecular weight from 33k to 150k MW in a closely related pyralid species, the Indian meal moth, *P. interpunctella*. Proteins extracted from eggs of *P. interpunctella* by the same procedure described in this study stimulated egg production in *O. insidiosus* fed artificial diet; however, a chloroform:methanol extract of the egg lipids had no effect (Ferkovich & Shapiro 2004a). Furthermore, in a separate study with *O. insidiosus*, in which total protein, RNA, and DNA were extracted from *Ephestia* eggs and bioassayed in diet, only the egg proteins stimulated the rate of oviposition when the three egg-extracted components were bioassayed in artificial diet (unpublished data).

Fecundity could be improved further with the addition of a higher concentration of the fraction that increases the rate of oviposition. Wheeler (1996) indicated that oogenesis is typically a nutrient-limited process and is initiated only if sufficient nourishment is taken for egg production. Adequate nourishment for *O. insidiosus* egg production is apparently not acquired during the nymphal stage because newly emerged adults that fed on the control Diet oviposited fewer eggs that those fed on whole *Ephestia* eggs. Only females that fed on Diet supplemented with fraction #16 oviposited significantly more eggs than the control Diet. This indicated that a specific nutrient or factor is required for egg production and is found in the protein component of the prey egg. The nature of factor, however, is unknown and awaits further purification and characterization. Protein extracts from whole eggs of *P. interpunctella* and a cell line derived from eggs of *P. interpunctella* (Ferkovich & Shapiro 2004 a & b) stimulated egg production of *O. insidiosus* females, but this is the first report of a specific protein fraction from prey eggs having oviposition-stimulating activity.

In view of a specific fraction having a positive effect on oviposition in *O. insidiosus*, we suggest that future research should focus on identifying the oviposition-enhancing material(s) from *Ephestia* eggs so that it can be more easily tested at various concentrations in the diet. Moreover, once the identity of the material is known, it may be possible to obtain it from a commercial source for supplementing the diet of *O. insidiosus*.

Acknowledgments

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References Cited

Barber, G. W. 1936. *Orius insidiosus* (Say), an important natural enemy of the corn earworm. U. S. Dept. of Agric. Tech. Bull. 504, 24 pp.

Carpenter, J. E., and P. Greany. 1998. Comparative development and performance of artificially reared vs. host-reared *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) wasps. Biol. Control 11: 203-208.

De Clercq, P., and D. DeGheele. 1992. A meat-based diet for rearing the predatory stinkbugs *Podisus maculiventri*s and *Podisus sagitta* [Het.: Pentatomidae]. Entomophaga 37(1): 149-157.

De Clercq, P., and D. DeGheele. 1993a. Quality assessment of the predatory bugs *Podisus maculiventris* (Say) and *Podisus sagitta* (Fab.) (Heteroptera: Pentatomidae) after prolonged rearing on a meat-based artificial diet. Biocont. Sci. and Tech. 3: 133-139.

De Clercq, P., and D. DeGheele. 1993b. Quality of predatory bugs of the genus *Podisus* reared on natural and artificial diets. Proc 7th workshop of the global IOBC working group "Quality control of mass reared arthropods" Rimini (1). 13-16 Sept.

De Clercq, P., F. Merlevede, and L. Tirry. 1998. Unnatural prey and artificial diets for rearing *Podisus maculiventris* (Heteroptera: Pentatomidae). Biol. Control 12: 137-142.

Ferkovich, S. M., J. A. Morales-Ramos, M. G. Rojas, H. Oberlander, J. E. Carpenter, and P. Greany. 1999. Rearing of ectoparasitoid *Diapetimorpha introita* on an artificial diet: supplementation with insect cell line-derived factors. BioControl 44: 29-45.

Ferkovich, S. M., and J. P. Shapiro. 2004a. Comparison of prey-derived and non-insect supplements on egg-laying of *Orius insidiosus* maintained on artificial diet as adults. Biol. Control 31: 57-64.

Ferkovich, S. M., and J. P. Shapiro. 2004b. Increased egg-laying in *Orius insidiosus* (Hemiptera: Anthocoridae) fed artificial diet supplemented with an embryonic cell line. Biol. Control 31: 11-15.

Funderburk, J., J. Stavisky, and S. Olsen. 2000. Predation of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in field peppers by *Orius insidiosus* (Hemiptera: Anthocoridae). Environ. Entomol. 29: 376-382.

McCaffrey, J. P., and R. L. Horsburgh. 1986. Biology of *Orius insidiosus* (Heteroptera: Anthocoridae): a predator in Virginia apple orchards. Environ. Entomol. 15: 994-998.

SHAPIRO, J. P., AND S. M. FERKOVICH. 2002. Yolk protein immunoassays (YP-ELISA) to assess diet and reproductive quality of mass-reared *Orius insidiosus* (Heteroptera: Anthocoridae). J. Econ. Entomol. 95: 927-935.

SHAPIRO, J. P., H. A. WASSERMAN, P. D. GREANY, AND J. L. NATION. 2000. Vitellin and vitellogenin in the soldier bug, *Podisus maculiventris*: Identification with monoclonal antibodies and reproductive response to diet. Arch. Insect Biochem. Physiol. 44: 130-135.

SHIRK, P. D., D. BEAN, A. M. MILLEMANN, AND V. J. BROOKES. 1984. Identification, synthesis, and characterization of the yolk polypeptides of *Plodia interpunctella*. J. Exp. Zool. 232: 87-98.

VAN LENTEREN, J. C., M. M. ROSKAM, AND R. TIMMER. 1997. Commercial mass production and pricing of organisms for biological control of pests in Europe. Biol. Control 10: 143-149.

VAN DER VEIRE, M., AND D. DEGHEELE. 1992. Biological control of the western flower thrips, *Frankliniella occidentalis* (Pergrande) (Thysanoptera: Thripidae), in glasshouse sweet peppers with *Orius* spp. (Hemiptera: Anthrocoridae). A comparative study between *O. niger* (Wolff) and *O. insidiosus* (Say). Biocontrol Sci. and Tech. 2: 281-283.

WEIRU, Z., AND W. REN. 1989. Rearing of *Orius sauteri* (Hemiptera: Anthrocoridae) with natural and artificial diets. Chinese J. Biol. Control 5: 9-12.

WHEELER, D. 1996. The role of nourishment in oogenesis. Annu. Rev. Entomol. 41: 407- 431.

WITTMEYER, J. L., AND T. A. COUDRON. 2001. Life table parameters, reproductive rate, intrinsic rate of increase, and estimated cost of rearing *Podisus maculiventris* (Heteroptera: Pentatomidae) on artificial diet. J. Econ. Entomol. 94(6): 1344-1352.

COURTSHIP OF THE TWO FEMALE MORPHS OF *MELITTOBIA DIGITATA* (HYMENOPTERA: EULOPHIDAE)

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ABSTRACT

Courtship of sib-mating *Melittobia digitata* Dahms, a parasitoid of solitary wasps and bees, is reviewed, described, and quantified for 125 virgins of the non-dispersing brachypterous female (BF) morph paired with 24 experienced males, and for 158 virgins of the dispersing macropterous female (MF) morph paired with 21 males. Males performed 1-5 courtship bouts with both morphs; about half of all successful matings in both morphs occurred after a single bout. Depending on number of bouts performed, mean courtship durations ranged from 47-268 sec for MFs and 59-277 sec for BFs. Courtship success rates were greater for BF couples (80%) than for MF couples (57%). Compared to BF couples, MF couples were more apt to undergo multiple bouts. Results are interpreted in the context of the morphs' life history and the costs/benefits of alternatives. Failure to initiate any courtship during the 15-min observation period (22% for MF pairs, 21% for BF pairs) appeared to be due to apparent lack of interest or to occasional male violence toward females. Possible explanations for the latter, including mistaken identity, odor contamination, and nutritional stress are discussed.

Key Words: polymorphism, sexual selection theory, alternative reproductive strategies, reproductive isolation, aggression, sib mating

RESUMEN

El cortejo de *Melittobia digitata* Dahms, parasitoide de avispas y abejas solitarias que se aparea con sus hermanos, se revisa, se describe, y se cuantifica para 125 vírgenes de la forma hembra braquíptera que no dispersa (HB) apareadas con 24 machos experimentados, y para 158 vírgenes de la forma hembra macróptera que dispersa (HM) apareadas con 21 machos. Los machos ejecutaron 1-5 sesiones de cortejo con las dos formas femenias; aproximadamente la mitad de las uniones exitosas en las dos formas ocurrió después de una sola sesión. Dependiente del número de sesiones implementadas, las duraciones promedias para el cortejo duraron entre 47-268 segundos para HMs y 59-277 segundos para HBs. La tasa de cortejo exitoso fue más alta para parejas HB (80%) que para parejas HM (57%). Comparadas con las parejas HB, las parejas HM solían ejecutar sesiones múltiples. Los resultados se interpretan en el contexto de la historia vital de los morfos y los costos/beneficios de las alternativas. La falta de iniciar cortejo durante el período de observación de 15 minutos (22% para parejas HM, 21% para parejas HB) pareciera ser por falta de interés o por violencia ocasional de los machos hacia las hembras. Explicaciones posibles para éste, incluyendo identidad errónea, contaminación de olor y estrés nutricional se discuten.
Translation provided by the authors.

Melittobia are small (ca. 1 mm) eulophid wasps that are ectoparasitic upon prepupae or pupae of various larger insects, particularly solitary wasps and bees. Upon discovering a potential host, a female stings it, then feeds on host hemolymph emanating from the wound(s); this enables her to develop and then lay dozens to hundreds of eggs on that host (Dahms 1984b). Extreme inbreeding characterizes this genus; sib mating is the rule, and as a result of haplodiploid sex determination (arrhenotoky), virgin females produce sons with whom they can mate (Dahms 1984b).

Melittobia are unusual in having polymorphic female forms (Fig. 1), as first described by Schmieder (1933). Under certain conditions (apparently nutritional— see Cônsoli & Vinson 2002, 2004), a small number of females (<30) develop more quickly than the rest, and emerge as short-winged, stout-bodied individuals. Each of these "brachypterous" (BF) females (termed "second form" by Schmieder, 1933) is born with a clutch of about 30 mature eggs (Cônsoli & Vinson 2002) that they immediately lay on their natal host soon after mating with an early-emerging brother. All later-developing females on the same host possess functional wings. These "macropterous" (MF) females (termed "type form" by Schmieder, 1933) have incompletely developed eggs that mature only after they have fed on a new host after dispersing (Cônsoli & Vinson 2002).

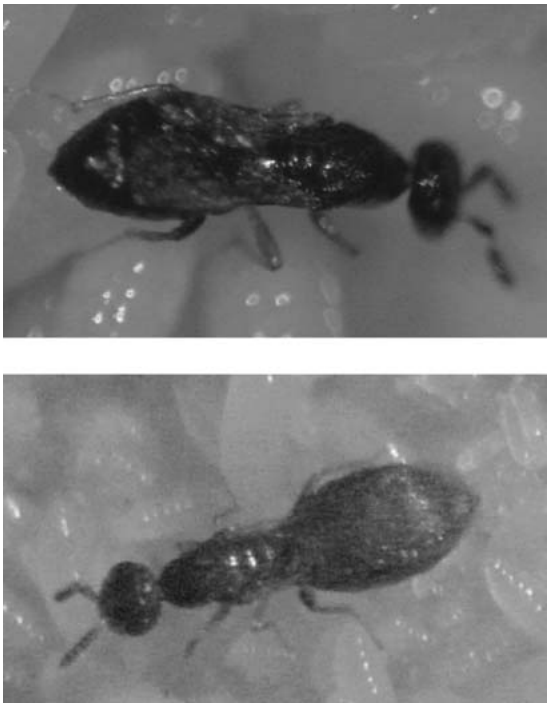

Fig. 1. Female morphs of *M. digitata*. Above: Brachypterous form [BF] (= second form of Schmieder 1933); Below: Macropterous form [MF] (= type form of Schmieder 1933). Body length of MF, 1.2mm.

Aspects of the courtship behavior of 10 of the 14 known *Melittobia* species have been reported by Assem (1975, 1976); Assem et al. (1982); Assem & Maeta (1978, 1980); Dahms (1973); Doroshina (1989); Evans & Matthews (1976); González (1985, 1994); González et al. (1996); Lapp (1994); and Varanda et al. (1984). Guided largely by chemical and tactile cues, the blind males perform an elaborate sequence of leg, wing, and antennal movements that vary from one species to another but are always surprisingly complex. Males of several *Melittobia* species appear to release a pheromone to which virgin MFs are strongly attracted (Cônsoli et al. 2002; Hermann et al. 1974; González et al. 1985; González et al. 1996; Matthews et al. 1985), often to the extent of forming a queue around a courting couple (Assem 1976). The male pheromone of *M. digitata* has been identified as *trans*-bergamotene (Cônsoli et al. 2002).

Further knowledge of the nature of courtship in *Melittobia* is desirable for a number of reasons. With few exceptions (González 1985, 1994; González et al. 1996; Lapp 1994), previous studies have involved only MF. As a practical matter, study of *Melittobia* mating rituals might identify behavioral characters useful for understanding species relationships where morphological traits alone leave some uncertainty about species boundaries (Assem et al. 1982; González et al. 1996); in one case, such information already has been pivotal (Evans & Matthews 1976). Because *Melittobia* increasingly are being used as model organisms to teach various concepts in the life sciences curriculum (e.g., Guinan et al. 2000; Matthews 1997, 1982; Matthews & Matthews 2003; Matthews et al. 1996; Pyle et al. 1997), basic biological information such as that in this study also will help teachers and students by supporting and underpinning curriculum materials.

The objectives of this study were to quantify and compare the courtship interactions for both morphs (MF and BF) of *M. digitata,* and to relate findings to other aspects of the life history.

Materials and Methods

Laboratory cultures of *Melittobia digitata* were started with individuals that were reared from field-parasitized nests of mud dauber wasps (*Trypoxylon politum* Say, Hymenoptera; Sphecidae). The parasitoids were maintained in continuous culture at 25°C and 75% RH on *T. politum* prepupae.

To obtain virgin females for the courtship trials, groups of female pupae (distinguished from males by the presence of eyes) were isolated. Because BFs develop somewhat faster than MFs (Cônsoli & Vinson 2002), to obtain them we isolated the first 20-30 pupae to develop on each parasitized mud dauber prepupa. All later-developing female pupae were of the macropterous morph.

At 24-48 h after eclosion, each female was placed with a randomly chosen male removed directly from laboratory stock cultures. Unlike females, males of *M. digitata* are not known to be polymorphic. By continually monitoring the stock cultures, we knew that the selected males were 1 to 3 days old; because males are well documented to mate readily and repeatedly (e.g., Assem et al. 1982; Dahms 1984), we presumed them all to be experienced.

Each male-female pair was placed in a deep well slide (8 mm diameter, 3 mm depth), capped with a glass cover slip, and illuminated with a fiber optic lamp. Interactions were observed at ambient temperatures (23°C ± 1°) and were recorded with a Sony digital video camera with an attached Macro-Zoom lens (18-108 mm). Data on pairing outcomes and durations of selected courtship components were subsequently transcribed from the video recordings. If no courtship activity occurred within 15 min, a trial was terminated. Between trials, slides were washed with 95% ethanol; new cover slips were used for each trial.

We recorded 158 pairings with MF and 125 pairings with BF. Individual males were used for 1-5 successive pairings; because females mate but

once, every trial used a different female. Statistical comparisons of various parameters for the two morphs used the student's *t*-test.

Results and Discussion

Overall, 124/158 (78%) of MF and 99/125 (79%) of BF began courting within 15 min after being placed with an experienced male. Among the 124 MF courting pairs, 71 females (57%) mated; among the 99 BF courting pairs, 79 females (80%) mated (Table 1). The difference in proportion of overall mating success was significant ($P < 0.001$). Bout-by-bout comparisons of mating success rates showed similar significantly greater success rates for BF pairs in bouts 1 ($P = 0.011$) and 2 ($P = 0.034$), but no differences between the proportions of each morph succeeding in bouts 3, 4, and 5. Every randomly assigned male mated at least once.

Initial Attraction

When a female responds to the male's odor by touching the side of his abdomen with her antennae, the male typically responds by turning toward her body and touching her side with his antennae. This is a female's first decision point. If she is not receptive, she attempts to move away, with her antennae held downward. If she is receptive, she becomes still. Approaching either posteriorly or laterally, the male then mounts her dorsum and usually turns first toward her posterior, then reverses and ultimately orients his body in a plane parallel to hers. He then moves forward and makes initial contact with his antennae. At this point, we defined a courtship bout as having begun.

Single- and Multiple-Bout Courtship

Qualitatively, courtship was essentially the same for both morphs and our description thus applies to both. During a courtship bout, the male holds the female by placing his front tarsi just behind her head, his middle tarsi on the sides of her metathorax aligned with her middle and hind legs, and his hind tarsi on her dorsal anterior abdomen with wings and abdomen raised, he begins rhythmically opening and closing his antennae in a more or less lateral plane. As he does so, his antennae contact the clubs of the female's antennal flagellae. During each closing stroke, he maneuvers her clubs into the ventral grooves on his scapes, where they are briefly embraced by the modified pedicel and a finger-like scape projection (the "digit" that inspired this wasp's specific epithet). During each opening stroke, her antennal clubs are released, thereby completing an antennal stroking cycle.

Concurrently, the male begins a rhythmic kicking-lifting-swinging motion with his hind legs. At the end of each antennal cycle, the male's hind legs kick rapidly outwards, lift upward and forward, and slowly swing around return to their original position on the female's abdomen. Concurrently, the male also lowers his wings slightly and begins to flutter them, and rests the tip of his arched abdomen on the dorsum of the female's abdomen.

Initial alternations of antennal stroking and leg kicking appear leisurely, but the pace of the alternation soon accelerates. Kicking becomes less vigorous and more like continuous quivering, as antennal movements tighten in scope and increase in tempo. During each succeeding antennal phase, the male antennae open less widely, and ultimately do not appear to open at all. Concurrently, wing fluttering becomes more intense.

In a final convulsive motion, the male stretches his abdomen backward and straightens his hind legs, effectively lengthening his body in a plane parallel to the female's body axis, and swings his middle legs forward to hit the back of the female's head, concluding the bout.

First-bout conclusion represents a second distinct female decision point. The female's behavior at this time directs the courtship's subsequent direction. If she stretches lengthwise, flattens her abdomen into a wedge-like profile, and exposes her genital aperture, the male immediately undertakes a distinctive "backing-up" behavior with an easily measurable duration. He comes into position, bends his abdomen forward under hers, inserts his aedeagus, and copulation ensues.

Table 1. Duration of successful courtships between *Melittobia digitata* makes and females of the tow morphological forms in relation to number of bouts performed. Total courtship duration is sum of individual bout lengths plus backup and copulation time. Values are means ± S.D.

Female Morphology	Total Courtship Duration (sec)					
	1 bout	2 bouts	3 bouts	4 bouts	5 bouts	All successful courtships
Macropterous (long winged)	47.2 ± 18.3 (n = 36)	85.6 ± 17.9 (n = 24)	146.2 ± 33.2 (n = 7)	201.5 ± 20.8 (n = 3)	267.8 (n = 1)	79.6 ± 50.2 (n = 71)
Brachypterous (short winged)	59.1 ± 24.2 (n = 45)	106.8 ± 27.6 (n = 29)	133.0 ± 24.1 (n = 2)	169.2 ± 31.2 (n = 2)	276.5 (n = 1)	84.0 ± 43.6 (n = 79)

If the female does not stretch and flatten (i.e., fails to signal receptivity), two possibilities arise. In the commonest outcome, a 'persistent' male does not back up nor dismount; instead, after a brief pause (<5 sec) he begins a second courtship bout (bout 2) with renewed slow and exaggerated antennal stroking. The other outcome occurs with a 'non-persistent' male. In this case, when the female fails to indicate receptivity, the male simply dismounts rather than beginning anew. In three cases of BF pairs a dismounted male immediately remounted the same female and began a new bout 1, but most often dismounted males moved away from the female without further interaction (compare flow charts at bout 1 in Figs. 2 and 3).

The fact that the females' behavior seems to determine the outcome of courtship is not surprising. However, the fact that *Melittobia* females wait until the conclusion of a complete bout sequence by the male (termed the "finale" by Assem et al. 1982) prior to indicating whether they are receptive or not is thought to be unique among the chalcidoid wasps. In other chalcidoid species so far studied, female receptivity may be indicated at varying points during the male's display, obviating the need for males to complete a full stereotyped display (Assem et al. 1982).

About half of all courtships that ultimately resulted in successful copulation occurred after only a single courtship bout (MF, 36/71; BF, 45/79); the other half required additional courtship, most commonly 1 more bout, rarely as many as 4 more (Figs. 2 and 3). With 2 bouts, the cumulative copulation success rate for both morphs increased dramatically (84.5% for MF pairings, 93.7% for BF pairings).

Assem and colleagues (1982) are the only other researchers to have described the courtship of *M. digitata* (their *M*. species 4). They used only macropterous females and did not systematically track pairing outcomes, nor did they record frequencies or outcomes of unsuccessful courtship interactions. For comparisons with unsuccessful courtship, they paired males with previously mated females, because *Melittobia* females will usually allow only a single copulation. Their descriptive data for 25 MF pairs differ slightly in terminology, but agree with our observations in all essential respects. Their average of 76.6 ± 9.6 sec for courtship duration and average of 25 leg-kick-lift (swing) cycles are both similar to our MF findings. They do not present data for courtships having more than one bout, and although we regularly observed multiple-bout courtships (Figs. 2 and 3), it is not clear whether they ever saw any.

Morphological Effects

Although essentially the same proportion of both morphs began courting, virgins of the BF morph that courted were more initially receptive to mating than were courting MF virgins. As the numbers above indicate, BF were more likely to require only a single courtship bout, and had a higher copulation success rate overall. Understandably, as the number of courtship bouts required for inducing receptivity in *M. digitata* goes up, courtship duration does also (Table 1). Depending upon the number of courtship bouts that preceded female receptivity signaling, a successful courtship with MF required 47-268 sec. With BF, again depending upon number of bouts, successful courtship lengths ranged from 59-277 sec.

Comparison of duration of first bouts of successful (female displays receptivity posture) versus unsuccessful (female fails to display receptivity) courting couples revealed that unsuccessful courtship durations were significantly shorter in BF couples ($P = 0.035$, $t = -2.238$, 14 *df* versus $P = 0.657$, $t = 0.446$, 64 *df* for MF couples). Thus for BFs, decisions about whether to copulate may relate to male bout duration, but not for MFs. Assem et al. (1982) mention that there was no difference between successful and unsuccessful courtship durations in any species they studied except for *M. clavicornis,* however, they did not compare the two morphs. Interestingly, successful and unsuccessful couples of both morphs did not differ in leg-kick-lift-swings/min in their first courtship bouts ($P = 0.76$, $t = 0.313$, 10 *df* for BF couples; $P = 0.613$, $t = 0.658$, 60 *df* for MF couples). Thus differences in first bout duration of successful and unsuccessful couples of the two morphs was not related to the rate of leg-kick-lift-swings.

Overall, successful courtship durations averaged slightly longer for the BF pairings (Table 1), but the difference was not statistically significant ($P = 0.26$, $t = -0.572$, 140 *df*). However, bout-by-bout comparisons showed that for BF pairs average successful bout durations were significantly longer for the first two bouts (bout 1 $P = 0.007$; bout 2 $P = 0.001$), but considerably shorter for the third and forth bouts (Table 1).

The longer overall average courtship durations for successful BF pairs may simply reflect the fact that BFs are demonstrably thickset and slow-moving in comparison to their slimmer, livelier MF siblings (see Fig. 1). This difference in female shape and agility, and male compensation for it, may also account for the finding that average copulation time for all successful BF couples was longer than for MF couples (6.6 ± 2.5 sec versus 5.0 ± 1.4 sec, $P < 0.001$, $t = 4.668$, 121 *df*) and all back-up times were less for males with BFs (4.6 ± 2.9 sec versus 6.6 ± 1.6 sec, $P < 0.001$, $t = -5.051$, 124 *df*). The longer average duration of successful BF courtships may also reflect a disparity in female receptivity thresholds. Males successfully copulating with BFs used on average 3 more leg kick-lift-antennal stroke cycles per bout than successful males courting MFs (27.5 versus 24.5). In addition, the relative pace of the leg-kick-lift-

swings/min was greater in successful MF couples (BF mean =36.3 ± 6.2, MF mean =39.5 ± 7.5; P = 0.005, t = -2.889, 148 *df*). Thus, males courting BFs used a greater number of leg-kick-lift cycles and performed them at a somewhat slower pace compared to males courting Mfs. This in combination with the longer average copulation time for BF couples resulted in the longer average courtship durations for BF couples compared to MF couples.

Courtship Success

In the course of their shared courtship reaction chain, both sexes have opportunities for choice. Females can signal decisions about male acceptance both at the initial encounter and after the male has mounted and completed a courtship display bout. Likewise, a male can decide whether to respond to a female's initial touch, and whether to persist or leave when a given bout does not result in female receptivity.

As noted above, the two morphs began courtship at about the same rate, but overall, BF couples had significantly higher courtship success rates over all bouts combined and for each of the first two bouts analyzed separately. Among all initial courtship pairings that failed to result in copulation, MF pairs quit about twice as often as BF pairs (43% versus 19%, Figs. 2 and 3). Of 124 MF pairs that began a first bout, only 36 successfully copulated at the conclusion of that bout (29%), compared to 45 of the 99 BF pairs (46%).

These differences may relate to the differing ecological roles of each female morph (Freeman & Ittyeipe 1976, 1982; González & Terán 2001). As the dispersive portion of the population, MFs emerge with undeveloped eggs. Their options include (1) to mate with their brothers (rarely with unrelated males, see below) and then disperse; (2) to disperse as a virgin to a new host and produce sons with which they can and do mate (e.g., Assem 1976; Dahms 1984; Schmieder 1933); or (3) to find both a new host and an unrelated male with which to mate. Options 2 and 3 likely are very uncommon in nature, since parasitized hosts normally always yield progeny of both sexes, and Abe et al. (2003a) has confirmed that all dispersing *M. australica* females are fertilized. Moreover, even if a virgin female somehow rejects one male's initial courtship attempt, she is likely to have other mating opportunities with the same or other sibling males in the same clutch.

In contrast, as the nondispersive portion of the population, each BF lays her eggs upon the remnants of the natal host, a limited resource that is already declining in nutritional quality due to feeding pressure (Cônsoli & Vinson 2002). Despite attempts to facilitate BF dispersal on foot to neighbor hosts in the laboratory, we have never observed a BF female to leave her natal host (unpubl. observ.) and we doubt that it ever occurs in nature. Thus BFs are in resource- and time-driven competition with each other for the success of their own offspring. Without the option of dispersing to new hosts, readily mating with clutch mates and rapidly ovipositing on the natal host would be strongly favored. Thus, it is perhaps not surprising that BFs mate more readily than MFs.

The difference in sex ratios of the early and later emerging *M. digitata* may also have relevance for the higher receptivity of BF. The very first progeny to emerge from a single female *M. digitata*-parasitized mud dauber consist of an average of 26.7 BF and 12.1 males (R. W. Matthews, unpubl.). Thus the initial sex ratio is much less female-biased than the final sex ratio will be after all the MFs have emerged. From a lone male's standpoint, additional mating opportunities with virgin BFs are far fewer than for MFs and the competition from brothers is relatively greater. From a BF's perspective there are far more potential male mating partners than needed since she will only mate one time, and has a host immediately at hand. Taken together, these life history variables also may help to explain why males paired with BFs performed both longer duration bouts and displayed a higher level of persistence into the second bout than males paired with MFs. BFs under these circumstances may require more "proof" of a male's genetic worth.

As noted above, courtship durations for the two morphs were not statistically different. However, there was a trend for males to perform more leg-kick-lift-swing cycles with BFs but BFs were more likely to require only a single courtship bout. Conversely, males performed fewer leg-kick-lift-swing cycles but more bouts with MFs. A possible scenario to explain these differences assumes that originally females appeared as only the macropterous morph. (While no phylogenetic analyses exist for *Melittobia* species, this assumption seems reasonable since macroptery is the most prevalent condition in the Chalcidoidea.) Males attempting to mate with MFs were (and are) under intense time pressure. Not only are they in fierce competition with their brothers (see Abe et al. 2005), but they are also racing the clock because any unmated females will begin to disperse as virgins after they are a few days old (unpubl. observ.). Due to natural variation, some MFs likely would be willing to copulate sooner than the average; the problem for a male is that he has no way to know in advance which females these might be.

Male courtship behavior might also be expected to vary, with some males "cheating" by attempting to reduce the number of leg-kick-lift-swing cycles, or inserting a finale partway along the series of leg-kick-lift-swing cycles. If the reduced effort was genetically based and proved acceptable to the female, such males would gain ad-

ditional time in which to court others; when it was not acceptable, they would lose only a few seconds, and could quickly resume the courtship. Such variation could lead to fewer leg-kick-lift-swing cycles per bout and an increase in the number of bouts required for success.

Compared to MF couples, time and performance pressures are reversed for BF couples. As noted above, for BFs time is of the essence. On the other hand, pressures to hurry along or "cheat" were/are much weaker for the males, due to a less extreme sex ratio and the lack of dispersal by BFs. Thus, in BF courtships, a gradual increase in the number of leg-kick-lift-swing cycles (resulting in an increased bout length) rather than fewer leg-kick-lift-swing cycle and more bouts might be favored.

Male Stability, Persistence, and Life Strategies

The world of the blind, flightless male of *M. digitata* is closed, violent, and highly competitive (see Abe et al. 2003a, 2003b, 2005; Hartley & Matthews 2003; and references therein), and he faces very real risks to life and limb from other males throughout his brief life. As a greatly outnumbered (average sex ratio, 3 males: 97 females [González & Matthews 2002]) male hurries to out-compete his brothers in the race to inseminate 500+ potential mates, success might be enhanced in many ways, but by any measure a male may gain an advantage through any reduction in courtship duration.

Assem et al. (1982) assert that successive courtships by a single male have very stable average durations (though they provide no data on the matter). Neither our study nor Assem's quantitatively compared virgin males with more experienced ones, but examination of our data subset for males with 2 successive single-bout courtships shows that for MF pairings (n = 9), the average duration of 42.7 sec with the first female was not different from the 43.4 sec duration with the second female (P = 0.835, t = -0.215, 8 df). Likewise, for BF pairings (n = 13), the average duration of 55.7 sec with the first female did not differ from the 68.1 sec duration with the second female (P = 0.133, t = -1.612, 12 df). These results suggest that experience does not increase the males' courtship efficiency.

Persistence may play an important role in the eventual success of a male. According to investment theory we can imagine that at the end of each unsuccessful bout, a male must choose between two alternatives: an investment in persistence in which he continues additional bouts with a so-far unreceptive female, or an investment in "playing the field" in which he always moves on after one bout. As noted earlier, although about half of all ultimately successful courtships with both morphs were consummated after a single bout, males paired with MFs succeeded in a single bout at a considerably lower rate than males paired with BFs. Males that continued through a second bout with initially unreceptive females dramatically increased their success rate, by 60% for males paired with MFs and 41% for males paired with BFs.

Pursuing the two alternatives, we assume a situation where virgin females are already queued up, so that searching time is minimized and courtship with a new individual can begin almost immediately after leaving the previous one. A successful single bout courtship with a MF takes about 47 s (Table 1), but in an initial encounter, a "field-playing" male has only a 51% chance of ultimately copulating. Since such a male cannot know in advance which "first date" will ultimately give rise to success, he would need to court 2 females (requiring an average of 94 s) to achieve an average of 1 copulation. On the other hand, a persistent male who does not leave after a single bout more than doubles his chances, and successful 2-bout courtship only requires an average of 86 s, including copulation. Thus for a male courting a MF, persistence is superior to playing the field. In addition, if a male stays in a "committed relationship" with a MF no matter how long it takes rather than leaving, his cumulative chance of ultimate success rises steadily. In contrast, the non-persistent field-player's chances of success remain at the initial 51%. Moreover, by persisting with a single female, a male's probability of encountering another aggressive male is nil, compared to what could ensue if he dismounted to play the field.

With BF pairs, a different picture emerges. A single-bout courtship takes somewhat longer than for a MF pair, about 59 s, but carries a higher potential success rate, so that a field-playing male on average will need to court fewer than two BF per copulation, at most 108 s. A persistent BF-courting male with success after 2 bouts requires 107 s, and increases his chances of success by less than 50%. Thus, time investment for the two alternative strategies is more similar in BF pairings than in MF pairings, relaxing selection for male persistence with the former. On the other hand, because BFs do not queue around males (unpubl. observ.), a male may require extra time and encounter increased risks from competing males in a search for another receptive female, factors that might favor persistence. It would be interesting to manipulate such factors experimentally to gain further insight into the evolution of alternative strategies.

When Courtship Goes Awry

All previous work on *Melittobia* courtship (including our own earlier work) ignores data for unsuccessful courtship pairings. However, it may be

instructive to examine the cases in which courtship goes wrong. As is evident from our data and the flow charts for both female morphs (Figs. 2 and 3), most instances of failure to court appeared to be a matter of non-attraction, at least during our relatively short observation period.

Among those 34 MF pairs that failed to court, 29 showed no sign of interest or receptivity on the part of one or the other sex during the trial (Fig. 2). Similarly, of those 26 BF pairs that did not court, 23 displayed no apparent interest (Fig. 3). The proportion of pairings in which females actively rebuffed the male's attempt to mount and appeared to refuse to cooperate was about the same for each morph, 26/158 (17%) for MF and 19/125 (15%) for BF.

Reasons for apparent lack of interest by the virgin females are unknown. Although it is possible that a deficiency in male pheromone production might have accounted for some of the female disinterest, we feel this is unlikely. Our males were 1-3 days old, and Cônsoli et al. (2002) found that male pheromone production peaked at 2 days post-emergence. Furthermore, each male used in our pairings successfully attracted and copulated with a virgin female on at least one occasion.

Alternatively, these cases might simply have been an artifact of the experimental situation. In our study, as in those before us, single individuals of each sex were confined together within a comparatively large, lighted space, whereas in their natural context, the sexes would emerge inside a crowded and dark host cocoon and have essentially unlimited time to get together. The relatively brief time allotted in our trials, plus possible physiological stress as a result of handling and manipulation, may have been contributing factors.

The remaining failures to court in our experiments involved cases of overt male aggression toward females (5/34 for MF pairs and 3/26 for BF pairs). In 2 of the trials with MFs, males killed their partner. With BFs, males also killed twice.

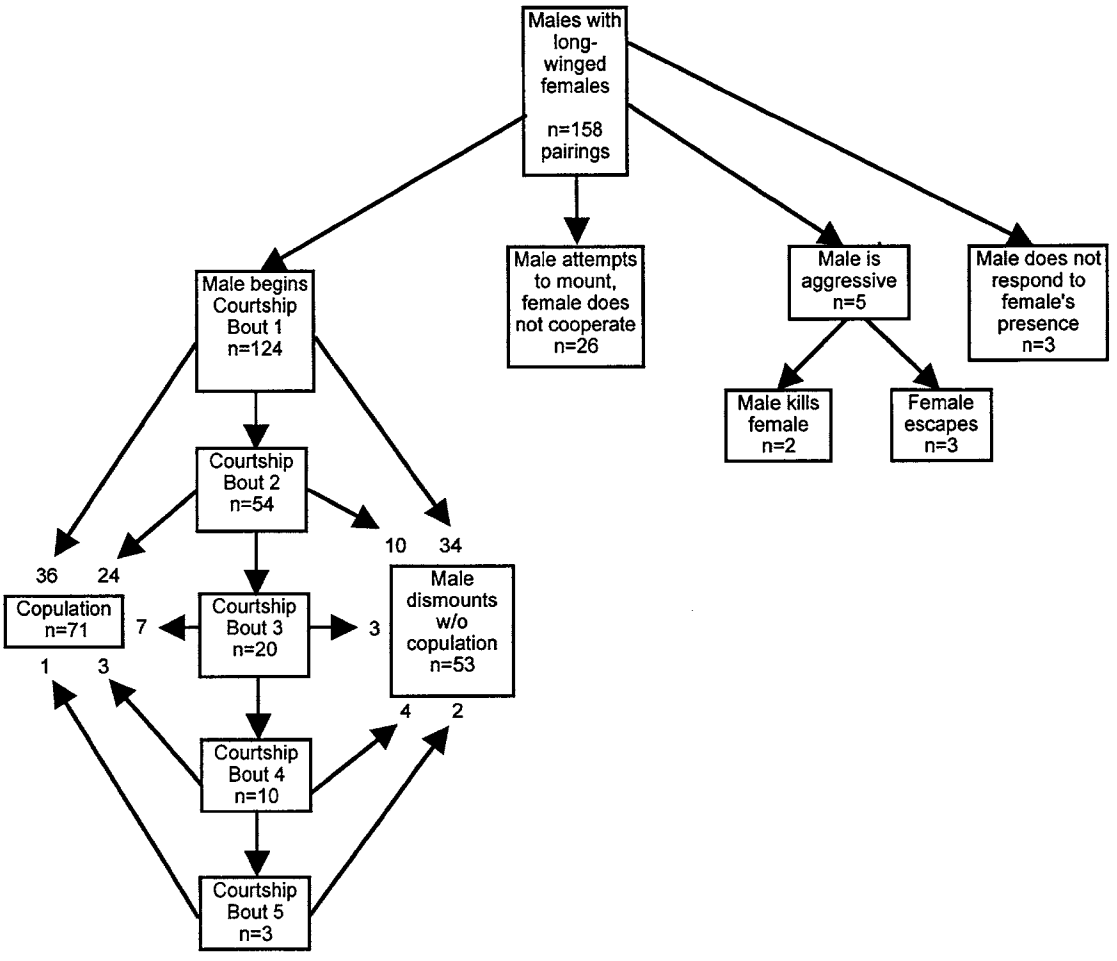

Fig. 2. Flow chart of the outcomes of 158 pairings of MF of *M. digitata*.

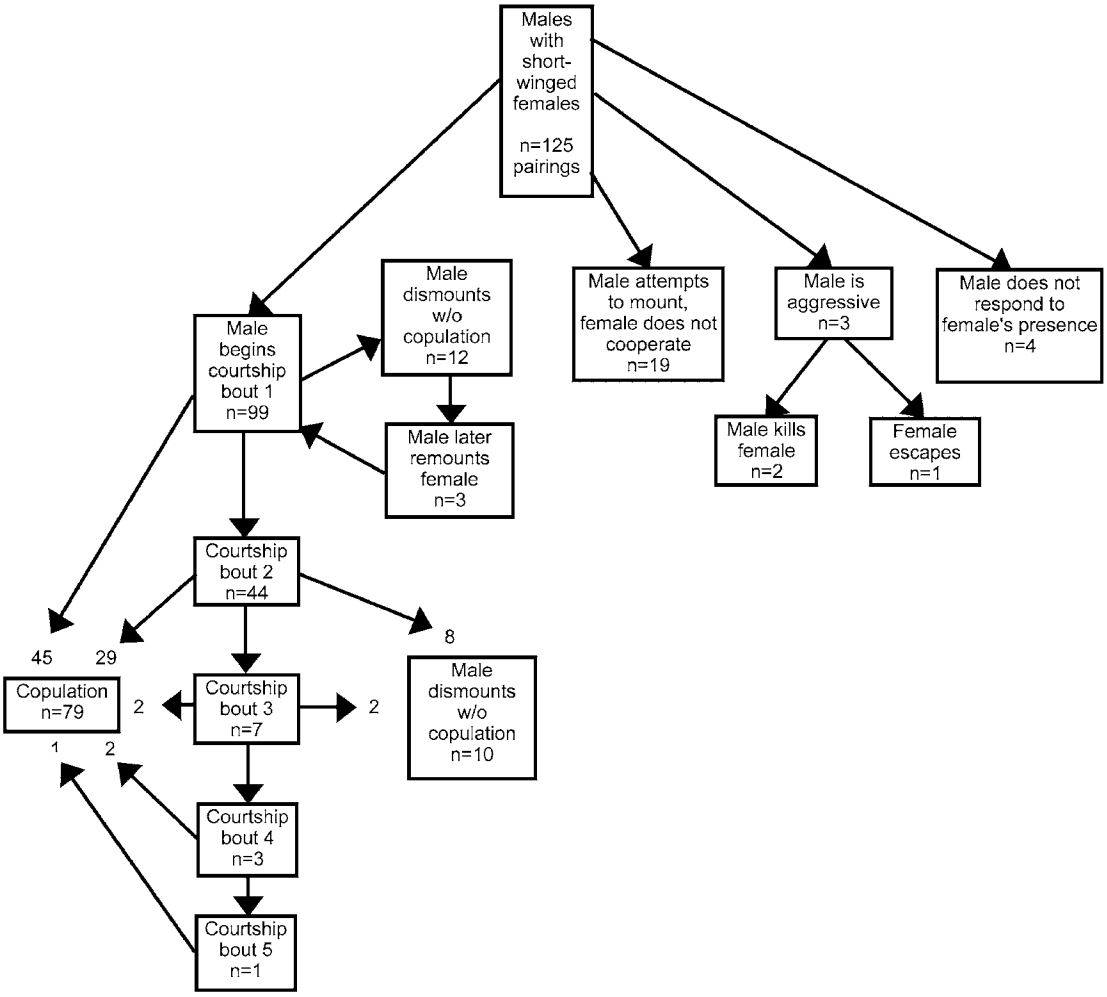

Fig. 3. Flow chart of the outcomes of 125 pairings of BF of *M. digitata*.

These attacks typically occurred after the male had mounted and appeared ready to begin a courtship bout. However, instead of moving forward to contact the female's antennae, the male instead would begin to chew at her neck or anterior thorax region. Our "killer males" mated normally on other occasions, but because each male in our study was used for only a limited number of trials, the question is left open as to whether these aggressive incidents represent an isolated (and perhaps environmentally influenced) fluke. Longer-term studies involving larger numbers of pairings with males that show such behavior would be valuable.

As noted above, male-male aggression often resulting in death is a characteristic of the genus *Melittobia*. However, the occurrence of aggressive actions toward conspecific virgin females is seldom mentioned in the literature and appears to vary between species. Balfour-Browne (1922) reported that males of *M. acasta* and *M. chalybii* (=*M. australica*) commonly killed females, but attributed this behavior to experimental conditions. Dahms (1984b) noted similar female killing behavior occasionally in his observations on *M. australica*. Neither previous study quantified the incidence of male violence to females.

The basis for such seemingly maladaptive behavior is unclear. Dahms (1984b) postulated that remnants of male odor might remain in the courtship chamber, stimulating male aggression that mistakenly became directed toward females. However, such a "laboratory artifact" is in fact the natural situation inside a host cocoon where, because males continually fight with one another, male odor is likely to be a constant part of the olfactory milieu.

Mistaken identity and/or inappropriate signaling might also be a factor. On occasion we have observed females behaving atypically. For example,

sometimes upon being antennated by a male, a female will retract her legs and assume an inert pupa-like form (unpubl. observ.). Such visually apparent weirdness would have little impact on sightless males within a dark host cocoon, but it might be accompanied by relevant (but as yet unknown) chemical, auditory, or tactile cues.

Nutritional stress provides a third not mutually exclusive explanation. Hermann (1971) mentioned that males of *M. chalybii* (=*M. australica*) 8 or more days old would "grasp and feed on" a receptive female. Matthews (1975) also noted males of this species chewing on the female victim during or after attack. Whether any nutritional benefit accrues to the male in these cases awaits further study.

Why Such Complex Courtship?

The elaborate courtship rituals observed in *Melittobia* parasitoids are reportedly some of the most intricate known in this large group of insects (Assem 1975). Since it is generally believed that *Melittobia* males never leave their natal host, and that all females are fertilized by their brothers (Dahms 1984b), the existence and maintenance of such complexity is somewhat perplexing.

Assem et al. (1982) hypothesized that the courtship might serve to prevent sperm depletion in males by spacing out copulations. However, this would not seem particularly relevant for males courting BFs, since there are rarely more than 30 females in a clutch of BF offspring. Additionally, Assem et al. (1982) raised the possibility that the leg-raising component may have arisen as a result of male-male competition and the need to fend off intruders, particularly other females attracted to the queue surrounding courting couples. Their argument would not apply particularly well to courtships with BFs, which behave sluggishly and show little tendency to queue around males. However, if, as suggested above, ancestral females only existed as the macropterous morph, the competitive nature of males, once evolved, may have persisted even after the BF morph appeared.

The role of courtship behavior in the maintenance of reproductive isolation may be of more importance to *Melittobia* than researchers have previously appreciated. Superparasitism in some *Melittobia* has been reported. Molumby (1996) discovered up to 5 *M. femorata* females (mean, 1.84) per host cell in a sample of 53 parasitized cells from 28 *Trypoxylon politum* hosts nests in Mississippi. Whether multiple species colonize a single host in nature is not known. In part this may reflect the fact that prior to 1984 it was believed that only one species (*M. chalybii*) existed in North America and another in the Old World (*M. acasta*). However, Schmeider (1933) mentioned possibly having more than one species in his studies and Dahms (1984a) recognized 8 species from North America. Indeed, on one occasion 3 different *Melittobia* species were found within a single field-collected host cell of *Trypoxylon politum* in Georgia (J. M. González, unpubl.). Furthermore, Matthews et al. (1985) note that *Melittobia* females are sometimes attracted to odors of males of other species. Thus the potential exists for multiple species to occur and interact in some localities. Even if relatively rare, cases of inter- and intraspecific host settling could provide a selective context favoring development and maintenance of elaborate courtship.

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References Cited

Abe J., Y. Kamimura, N. Kondo, and M. Shimada. 2003a. Extremely female-biased sex ratio and lethal male-male combat in a parasitoid wasp, *Melittobia australica* (Eulophidae). Behav. Ecol. 14: 34-39.

Abe J., Y. Kamimura, H. Ito, H. Matsuda, and M. Shimada. 2003b. Local mate competition with lethal male combat: Effects of competitive asymmetry and information availability on a sex ratio game. J. Evol. Biol. 16: 607-613.

Abe J., Y. Kamimura, and M. Shimada. 2005. Individual sex ratios and offspring emergence patterns in a parasitoid wasp, *Melittobia australica* (Eulophidae), with superparasitism and lethal combat among sons. Behav. Ecol. Sociobiol 57: 366-373.

Assem, J. van den. 1975. Temporal patterning of courtship behaviour in some parasitic Hymenoptera, with special reference to *Melittobia acasta*. J. Entomol. (A) 50: 137-146.

Assem, J. van den. 1976. Queue here for mating: Waarnemingen over het gedrag van ongepparde *Melittobia* wijfjes ten opzichte van een mannelijke soortgenoot. Entomol. Berich. 36: 74-79.

Assem, J. van den, and Y. Maeta. 1978. Some observations on *Melittobia* species (Hymenoptera, Chalcidoidea-Eulophidae) collected in Japan. Kontyû 46: 264-272.

Assem, J. van den, and Y. Maeta. 1980. On a fourth species of *Melittobia* from Japan. Kontyû 48: 477-481.

Assem, J. van den, H. A. J. in den Bosch, and E. Prooy. 1982. *Melittobia* courtship behavior: A comparative study of the evolution of a display. Netherlands J. Zool. 32: 427-471.

Balfour-Browne, F. 1922. On the life-history of *Melittobia acasta* Walker, a chalcid parasite of bees and wasps. Parasitology 14: 349-370.

Cônsoli, F. L., and S. B. Vinson. 2002. Clutch size, development and wing morph differentiation of *Melttobia digitata* Dahms (Hymenoptera: Eulophidae). Entomol. Exp. Appl. 102: 135-143.

CÔNSOLI, F. L., AND S. B. VINSON. 2004. Wing morph development and reproduction of the ectoparasitoid *Melittobia digitata*: nutritional and hormonal effects. Entomol. Exp. Appl. 112: 47-55.

CÔNSOLI, F. L., W. J. WILLIAMS, S. B. VINSON, R. W. MATTHEWS, AND M. F. COOPERBAND. 2002. *Trans*-bergamotenes—male pheromone of the ectoparasitoid *Melittobia digitata*. J. Chem. Ecol. 28: 1675-1689.

DAHMS, E. C. 1973. The courtship behaviour of *Melittobia australica* Girault, 1912, (Hymenoptera: Eulophidae). Mem. Queensland. Mus. 16: 411-414.

DAHMS, E. C. 1984a. Revision of the genus Melittobia (Chalcidoidea; Eulophidae) with the description of seven new species. Mem. Queensland Mus. 21: 271-336.

DAHMS, E. C. 1984b. A review of the biology of species in the genus *Melittobia* (Hymenoptera: Eulophidae) with interpretations and additions using observations on *Melittobia australica*. Mem. Queensland Mus. 21: 337-360.

DOROSHINA, L. P. 1989. Adapative features of *Melittobia acasta* (Chalcidoidea, Eulophidae), a parasite of solitary bees. Zoologich. Zhur. 68: 60-69.

EVANS, D. A., AND R. W. MATTHEWS. 1976. Comparative courtship behavior in two species of the chalcid wasp *Melittobia* (Hymenoptera: Eulophidae). Anim. Behav. 24: 46-51.

FREEMAN, B. E., AND K. ITTYEIPE. 1976. Field studies on the cumulative response of *Melittobia* sp. (*Hawaiiensis* complex) (Eulophidae) to varying host densities. J. Anim. Ecol. 45: 415-423.

FREEMAN, B. E., AND K. ITTYEIPE. 1982. Morph determination in *Melittobia*, a eulophid wasp. Ecol. Entomol. 7: 355-363.

GONZÁLEZ, J. M. 1985. Studies on courtship and sexual communication in *Melittobia* parasitic wasps (Hymenoptera: Eulophidae). M.Sc. thesis, University of Georgia, Athens, Georgia. 57 pp.

GONZÁLEZ, J. M. 1994. Taxonomía, biología y comportamiento de avispas parasíticas del género *Melittobia* Westwood (Hymenoptera: Eulophidae) en Venezuela. Ph. D. Thesis, Universidad Central de Venezuela, Maracay, Aragua. 118 pp.

GONZÁLEZ, J. M., AND R. W. MATTHEWS. 2002. Life history development and sex ratio of *Melittobia australica* and *M. digitata* (Hymenoptera: Eulophidae) on *M. rotundata* (Hymenoptera: Megachilidae) and *Trypoxylon politum* (Hymenoptera: Sphecidae). Great Lakes Entomol. 35: 85-91.

GONZÁLEZ, J. M., AND J. B. TERÁN. 2001. Dispersión, busqueda y acceso al hospedador por *Melittobia acasta* (Hymenoptera: Eulophidae). Bol. Cent. Inv. Biol L.U.Z. 35: 52-64.

GONZÁLEZ, J. M., R. W. MATTHEWS, AND J. R. MATTHEWS. 1985. A sex pheromone in males of *Melittobia australica* and *M. femorata* (Hymenoptera: Eulophidae). Florida Entomol. 68: 279-286.

GONZÁLEZ, J. M., R. W. MATTHEWS, AND J. B. TERÁN. 1996. Cortejo en las avispas parasitoides *Melittobia acasta* and *Melittobia australica* (Hymenoptera: Eulophidae). Rev. Biol. Trop. 44: 687-692.

GUINAN, J. A., R. W. MATTHEWS, AND J. R. MATTHEWS. 2000. Courtship reaction chains and mate attraction: A two-part laboratory activity using WOW-Bugs, a new model insect, pp. 380-404 *In* S. J. Karcher [ed.], Tested Studies for Laboratory Teaching. Vol. 21. Proc. 21st Workshop/Conf. Assoc. Biol. Lab. Ed.

HARTLEY C. S., AND R. W. MATTHEWS. 2003. The effect of body size on male-male combat in the parasitoid wasp *Melittobia digitata* (Hymenoptera: Eulophidae). J. Hymenop. Res. 12: 272-277.

HERMANN, L. D. 1971. The mating behavior of *Melittobia chalybii* (Hymenoptera: Eulophidae). M.Sc. Thesis, University of Georgia, Athens, Georgia. 52 pp.

HERMANN, L. D., H. R. HERMANN, AND R. W. MATTHEWS. 1974. A possible calling pheromone in *Melittobia chalybii* (Hymenoptera: Eulophidae). J. Georgia Entomol. Soc. 9: 17.

LAPP, S. A. 1994. The mating system of *Melittobia* parasitic wasps: Fluctuating asymmetry, sexual selection, and comparative courtship behavior. M.Sc. Thesis, University of Georgia, Athens, Georgia. 63 pp.

MATTHEWS, R. W. 1975. Courtship in parasitic wasps, pp. 66-86 *In* P. Price [ed.], Evolutionary Strategies of Parasitic Insects and Mites. Plenum, New York.

MATTHEWS, R. W. 1982. Courtship of *Melittobia* wasps, pp. 162-166 *In* J. R. Matthews and R. W. Matthews [eds.], Insect Behavior: A Sourcebook of Laboratory and Field Exercises. Westview, Boulder, Colorado.

MATTHEWS, R. W. 1997. Weird wonderful WOWBugs. Carolina Tips 60: 9-11.

MATTHEWS, R. W., AND J. R. MATTHEWS. 2003. Courtship and mate attraction in parasitic wasps, pp. 59-72 *In* B. J. Ploger and K. Yasukawa, [eds.], Exploring Animal Behavior in Laboratory and Field. Academic Press, New York.

MATTHEWS, R. W., J. YUKAWA, AND J. M. GONZÁLEZ. 1985. Sex pheromones in male *Melittobia* parasitic wasps: Female response to conspecific and congeneric males of 3 species. J. Ethol. 3: 59-62.

MATTHEWS, R. W., T. R. KOBALLA, JR., L. R. FLAGE, AND E. J. PYLE. 1996. WOWBugs: New Life for Life Science. Riverview Press, Athens, GA. 318 pp.

MOLUMBY, A. 1996. The evolutionary ecology of a gregariously nesting wasp, *Trypoxylon politum*. Ph.D. Dissertation, Univ. of Chicago.

PYLE, E. J., T. R. KOBALLA JR., R. W. MATTHEWS, AND L. R. FLAGE. 1997. Bugs in the laboratory: Decisions, decisions. . . or the lady or the tiger? Sci. Activ. 34: 25-30.

SCHMIEDER, R. G. 1933. The polymorphic forms of *Melittobia chalybii* Ashmead and the determining factors involved in their production, (Hymenoptera: Chalcidoidea, Eulophidae). Biol. Bull. Marine Biol. Lab., Woods Hole 65: 338-352.

VARANDA, E. A., C. S. TAKAHASHI, A. E. E. SOARES, AND L. A. DE OLIVEIRA CAMPOS. 1984. Considerations on the courtship and copulation behavior, sex ratio and life cycle of *Melittobia hawaiiensis* (Hym: Eulophidae). Rev. Brasil. Genet. 7: 65-72.

FEEDING AND SURVIVORSHIP OF BLUEBERRY MAGGOT FLIES (DIPTERA: TEPHRITIDAE) ON PROTEIN BAITS INCORPORATED WITH INSECTICIDES

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ABSTRACT

Laboratory feeding trials evaluated fly survivorship on six insecticides (acetamiprid, clothianidin, deltamethrin, fipronil, imidacloprid, and spinosad) incorporated at 4, 40, and 400 ppm in protein baits. Higher concentrations of insecticides resulted in increased fly mortality. At all concentrations of insecticides in baits, except those on deltamethrin, there was a significantly higher mortality 4 d after the initial feeding, compared with flies that fed on a control bait. The presence of clothianidin or imidacloprid in baits led to significantly less feeding compared with a control bait without insecticide. There were no feeding deterrent effects of bait containing either fipronil or spinosad compared with a control bait without insecticide. Exposure of flies to fresh bait containing 40 ppm of acetamiprid, clothianidin, or imidacloprid, resulted in significantly more flies becoming knocked down than the control. Baits containing 40 ppm of fipronil or spinosad resulted in higher levels of fly mortality than baits containing either neonicotinoids (acetamiprid, clothianidin, or imidacloprid) or no insecticide for trials with fresh and 1-d-old bait with unlimited exposure. At the rates tested baits containing deltamethrin resulted in no fly knockdown and always had the lowest mortality of any insecticide treatment. The tradeoffs between insecticides capable of knockdown and mortality are discussed as they relate to management of *R. mendax*.

Key Words: *Rhagoletis mendax*, acetamiprid, clothianidin, fipronil, imidacloprid, spinosad

RESUMEN

La sobrevivencia de la mosca, *Rhagoletis mendax*, contra seis insecticidas (acetamiprid, clothianidin, deltamethrin, fipronil, imidacloprid, y spinosad) incorporados a 4, 40, y 400 ppm en cebos de proteina fue evaluada en pruebas de alimentación en el laboratorio. Las concentraciones mas altas de insecticidas resultaron en un aumento de la mortalidad de las moscas. En todas las concentraciones de los insecticidas en cebo, menos aquellas tratadas con deltamethrin, hubo una mortalidad significativamente mas alta 4 d después de la alimentación inicial, comparada con las moscas que se alimentaron sobre el cebo de control. La presencia de clothianidin o imidacloprid en el cebo resulto en una alimentación significativamente menor comparada con el cebo de control sin insecticida. No hubo ningún efecto detrimental en la alimentación del cebo que tenia fipronil o spinosad comparado con el cebo de control sin insecticida. La exposición de las moscas al cebo fresco con 40 ppm de acetamiprid, clothianidin o imidacloprid, resulto en significativamente mas moscas derribadas que en el control. Los cebos con 40 ppm de fipronil o spinosad resultaron en un nivel mas alto de la mortalidad de moscas que en los cebos con neonicotinoids (acetamiprid, clothianidin, o imidacloprid) o sin insecticida para las pruebas con cebo fresco y de cebo de un dia con exposición sin limite. A las tasas de insecticidas probadas, ningún mosca fue afectada en la prueba de cebo con deltamethrin y siempre tenian la menor mortalidad que cualquier otro tratamiento de insecticidas. Se commentan sobre los factores de los insecticidas con la capacidad para un efecto de noqueo de las moscas versus un insecticida que mata las moscas en relación al manejo de *R. mendax*.

The blueberry maggot fly, *Rhagoletis mendax* Curran, is a serious pest of lowbush and highbush blueberries, *Vaccinium angustifolium* Aiton and *V. corymbosum* L., respectively, in the northeastern United States and Atlantic Provinces of Canada. In areas not infested with *R. mendax* there is zero-tolerance for maggot presence. As a result, growers exporting fruit to non-infested areas of

Canada must participate in a Blueberry Certification Program (Canadian Food Inspection Agency 1999).

This certification program mandates following either a calendar-based or an integrated pest management (IPM) spray program. A calendar-based approach requires growers to start spraying insecticides within 10 d of the first detection of an adult fly in the area, and continue spraying at 7- to 10-d intervals until the end of harvest. An IPM-spray program requires growers to monitor the presence of adults with traps baited with ammonium acetate. A recommended insecticide should be applied within 5 d of the date of capture of a single fly in any one of the monitoring traps, followed by a second spray 7-10 d later. This spray sequence should be repeated for each subsequent fly detection until the end of harvest. Many blueberry growers use one of these spray regimens, but there are several alternative strategies that have been investigated.

Rhagoletis flies can be controlled and managed by a variety of insecticides and application methods. Broad-spectrum insecticides, such as organophosphates and carbamates, have been applied in ultra low volume sprays, where contact and feeding toxicity of small droplets can cause fly mortality (Mohammad & Aliniazee 1989; Hu et al. 2000). The enactment of the Food Quality Protection Act (FQPA) (1996) has placed severe restrictions on the use of these broad-spectrum insecticides, and future management of *Rhagoletis* flies will involve the use of insecticides that are not impacted by FQPA reassessment. Many of these new compounds have little or no contact toxicity; therefore, they are often incorporated into a bait station or bait spray, in which mortality results after flies ingest significant quantities of insecticide.

Painted spheres baited with ammonia compounds are highly attractive to tephritids and have been developed as bait stations. Spheres were first coated with a sticky material to trap flies (Prokopy 1975), but the need for decreased deployment and handling time necessitated finding an insecticide replacement (Duan & Prokopy 1995b). Studies evaluating the effects of insecticides, which were incorporated into the paint and sugar matrix that coated the surface of spheres, have been performed for *Anastrepha ludens* (Loew) (Prokopy et al. 2000b); *Ceratitis capitata* (Wiedemann) (Hu et al. 1998); and *R. mendax* and *R. pomonella* (Walsh) (Duan & Prokopy 1995b; Liburd et al. 1999; Ayyappath et al. 2000; Stelinski et al. 2001). Comparisons between baited spheres and azinphos-methyl sprays in a commercial apple orchard showed similar reductions in populations of *R. pomonella* (Prokopy et al. 2000a). However, in commercial blueberry fields insecticidal spheres are not currently used because of the deployment density, lack of attractive selective lure, associated costs of products (i.e., spheres and residue extending agents), and labor requirements (i.e., monitoring and applying insecticides to spheres) (Barry et al. 2004).

Another alternative to broad-spectrum sprays are protein bait sprays which contain ammonia-based attractants, a feeding stimulant such as sucrose, and an insecticide. Protein bait sprays have been used to control outbreaks of *Anastrepha ludens*, *Bactrocera dorsalis* (Hendel), and *Ceratitis capitata* since the 1950s in the United States (Steiner 1952; Moreno & Mangan 2003). However, development and evaluation of protein bait sprays on *R. mendax* has begun only recently. Protein and ammonia-based attractants have been evaluated on *Anastrepha* spp. (Moreno & Mangan 2003), *B. cucurbitae* (Coquillett) (Fabre et al. 2003), *B. dorsalis* (Cornelius et al. 2000), *R. cerasi* (L.) (Katsoyannos et al. 2000), and *R. pomonella* (Duan & Prokopy 1992). Different concentrations of sugar feeding stimulants have been tested on *A. suspensa* (Loew) (Sharp & Chambers 1984), *R. pomonella* (Duan & Prokopy 1993), and *R. mendax* (Barry & Polavarapu 2004).

Dowell (1994) outlined alternatives to a malathion bait spray, which had been the preferred method in eradicating incipient infestations of *C. captitata*. Further development of a replacement has led to evaluation of insecticides classified as reduced risk. One compound that has already been incorporated into a bait spray for tropical and sub-tropical tephritid pests is spinosad, which was developed from the bacterium *Saccharopolyspora spinosa* Mertx and Yao. Feeding on baits containing spinosad has resulted in high mortality for *A. ludens* (Prokopy et al. 2000b), *A. suspensa* (King & Hennessey 1996), *B. cucurbitae* (Prokopy et al. 2003), and *C. capitata* (Peck & McQuate 2000; Vargas et al. 2001; Barry et al. 2003). Trials assessing toxicity have occurred for several of the non-organophosphate and non-carbamate compounds, such as deltamethrin, imidacloprid, and spinosad, on *R. pomonella* (Duan & Prokopy 1995a; Hu et al. 2000; Bostanian & Racette 2001; Reissig 2003) and acetamiprid, deltamethrin, fipronil, and imidacloprid on *R. mendax* (Barry et al. 2004). The reduced risk insecticide clothianidin, a neonicotinoid, has not been evaluated on any tephritid species.

Our goal was to identify the most effective concentrations of insecticides present in bait that resulted in knockdown, mortality, and had the least feeding deterrence on *R. mendax*.

Materials and Methods

Insects

Infested blueberries were collected near Chatsworth, NJ, in the summer of 2002 and 2003. The rearing procedures of Ayyappath et al. (2000)

were used to obtain adult *R. mendax*. Briefly, infested berries were placed over moist sand for larvae to drop and pupate. Puparia were sifted from sand three-five weeks later and kept in a screenhouse. Puparia were transferred to an incubator on 1 November 2002 and 2 November 2003, at 6°C with a photoperiod of 12:12 (L:D) to complete diapause. On 27 March 2003 and 30 March 2004 puparia were placed at 8°C. Periodically groups of puparia were transferred from 8 to 15°C for approximately 8 d and then transferred to an incubator at 25°C with a photoperiod of 16:8 (L:D) until adult emergence, which occurred 25-45 d later. Adult flies were kept at 22°C and were provided a diet of sucrose and water (i.e., protein-starved). Flies used in assays were 7-13 d-old and allowed to acclimatize to experimental conditions in the laboratory for several hours before trials commenced.

Feeding Assay—Feeding for 10 s

In the laboratory (21-23°C), a no-choice feeding test was used to evaluate survivorship of *R. mendax* on a control bait with baits containing three concentrations (4, 40, and 400 ppm or 0.0004, 0.004, and 0.04% [AI], respectively) of six insecticides: acetamiprid (technical, 30% [AI]; Cerexagri, King of Prussia, PA), clothianidin (technical, 49.17% [AI]; Arvesta, San Francisco, CA); deltamethrin and imidacloprid (technical 99.1, and 98.9% [AI], respectively; Bayer, Kansas City, MO); fipronil (technical 88% [AI]; Aventris Crop Science, Research Triangle Park, NC); and spinosad (technical, 90.4% [AI]; Dow AgroSciences, Indianapolis, IN). Solutions of each insecticide concentration were prepared by weighing the appropriate amount of technical and then adding it to the corresponding 1:3-mixture of SolBait (prepared as a 2× concentrate, USDA-ARS, Weslaco, TX) and water. (A 1:3-mixture corresponds to a 1:4 mixture of GF-120 Fruit Fly Bait [Dow AgroSciences] to water.) After preparing the highest concentration, serial dilutions with a 1:3-mixture of SolBait were used to obtain mixtures with lower concentrations of insecticides. The control was a 1:3-mixture of SolBait to water containing no insecticide.

One 10-µl droplet of bait was placed on a white plastic lid (5.5 cm in diameter) located on top of a plastic cylinder (4 cm in diameter, 4 cm in height) in the center of a Plexiglas cage (30 cm × 30 cm × 30 cm). A fly was transferred to this lid and placed next to the droplet. After feeding on a droplet for 10 seconds, the fly was removed from the lid and placed inside a plastic cylinder (5 cm in diameter, 8.5 cm in height) containing water and sucrose. Flies that fed less than 10 seconds were discarded, unless it was determined that after the initial feeding a fly became incapable of feeding as a result of the insecticide (i.e., knockdown). A total of 30 flies were evaluated with the control and for each of three concentrations of insecticide (except deltamethrin which was not evaluated at 4 ppm).

Flies were assessed for knockdown (i.e., immobile or incapable of walking) 1 h after the 10-s feeding. The number of dead, active, and incapacitated flies was recorded after 1, 2, 3 and 4 d. Flies were characterized as dead if there was no presence of visible body movement (i.e., no leg twitch), active if able to walk, and incapacitated if incapable of walking (Hu et al. 2000; Reissig 2003). The number of living flies is represented by the sum of active and incapacitated flies.

Feeding Assay—Feeding for 5 min

In the laboratory (21-23°C), a no-choice test was used to evaluate feeding propensity of female *R. mendax* on protein bait containing 40 ppm of insecticide. Treatments were prepared by the methods described in the 10-s assay and included a control bait (without insecticide), clothianidin, fipronil, imidacloprid, and spinosad. One 10-µl droplet of a treatment was placed on a silk ficus leaf (Michaels, Irving, TX) that was placed on top of a plastic cylinder (4 cm in diameter, 4 cm in height) in the center of a Plexiglas cage (30 × 30 × 30 cm). Silk leaves were preferred to blueberry leaves because of the presence of chemical cues in the latter. One fly was transferred to the leaf within 1 cm of the droplet. Each feeding trial ended after 5 min if a fly was still present on a leaf or when a fly left a leaf after 5 s. (Flies that left a leaf in less than 5 s were not counted because they were believed to be in an agitated state.) In addition, all flies had to feed a minimum of 1 s on the droplet.

The amount of time that a fly spent feeding on a droplet was recorded. Flies were assessed for knockdown 1 h after feeding. Mortality was recorded 1 and 4 d after feeding. Each fly was tested only once. A total of 28 replicates were completed for fly feeding and 20 replicates were completed for knockdown and mortality. One replicate was completed after a female fly had been tested on four protein baits incorporated with different insecticides and the control bait.

Exposure—4 h

Survivorship and knockdown of flies was assessed to blueberry bushes treated with insecticidal baits. A control bait and four baits containing 40 ppm insecticide of clothianidin, fipronil, imidacloprid, and spinosad, were prepared by the methods described in the 10-s Feeding Assay. Bait was applied with a handheld sprayer (30 Gunjet; Spraying Systems Co., Wheaton, IL) to deliver three 1-ml squirts at 30 psi to each of four three-yr-old blueberry bushes. This rate is equivalent to 9 liters/ha (0.95 gallons/acre). Three branches

(10-15 cm in length) were removed from each bush and placed inside a 250-ml Erlenmeyer flask in a Plexiglas cage (30 × 30 × 30 cm) that contained 20 flies (10 male, 10 female). The flask and branches were removed after 4 h. Flies were assessed for knockdown 3 h after introduction of treated branches and for mortality after 24 and 48 h. A total of 4 replicates were completed.

Unlimited Access—Fresh and 1-d-old bait

A no-choice assay evaluated fly mortality to bait containing the following insecticides: acetamiprid, clothianidin, fipronil, imidacloprid, and spinosad. Bait was prepared by adding enough technical insecticide to obtain 40 ppm [AI] in a mixture with GF-120 Fruit Fly Bait blank that did not contain spinosad (Dow AgroSciences). The control contained bait without the addition of insecticide. For each treatment, one 10-µl droplet of bait was applied to 60 highbush blueberry leaves. Half of these leaves were removed within 10 min of application for use in fresh assays and the other half remained on bushes for 24 h before being collected. Three treated leaves of the same bait were placed inside each of ten 1-liter plastic containers with a screened lid, which contained a moist cotton ball. Five flies were then placed in each container, which constituted a replicate. Flies were assessed for knockdown after 1 h and mortality 24 and 48 h after the start of exposure. Ten replicates were completed for fresh and 1-d-old bait. This experiment occurred in the laboratory where temperature was 21-23°C.

Statistical Analyses

Knockdown and survivorship data from the 10-s Feeding Assay are presented in tabular and graphical form, respectively. For this feeding assay, comparisons also were made between the control and each treatment with multiple chi-square tests after Bonferroni correction for the number of flies living versus dead after 4 d. In the 5-min assay feeding, duration was log transformed and analyzed by Fisher's least significant different (LSD) tests ($P = 0.05$). Knockdown and mortality were analyzed by multiple chi-square tests after Bonferroni correction. Prior to analysis of variance (ANOVA), mortality and knockdown were arcsine-square root transformed in both the 4-h exposure assay and the unlimited access assays (SAS Institute 1999). Means were separated by Fisher's LSD tests ($P = 0.05$).

Results

Feeding Assay—10 s

Insecticide type and concentration resulted in different survivorship of living (active + incapacitated) and active flies (Figs. 1 and 2, respectively). After 4 d, 97% of flies fed the control bait were living, which was significantly higher than all treatments except 4 ppm of acetamiprid, clothianidin, and imidacloprid, and 40 and 400 ppm of deltamethrin (χ^2, with Bonferroni correction, $P = 0.05$). Greater than 90% of flies were living 4 d after feeding on bait containing 4 ppm of acetamiprid, clothianidin, and imidacloprid (Fig. 1A-C); whereas less than 10% were living after feeding on baits with the same concentration of fipronil and spinosad (Fig. 1E, F).

Four days after feeding on bait containing 400 ppm of insecticide, there were 13, 43, and 87% flies categorized as living for treatments of clothianidin, acetamiprid, and deltamethrin, respectively (Fig. 1A, B, D). At 400 ppm of fipronil, spinosad, and imidacloprid in baits it took 1, 3, and 4 d after treatment to reach 0% survivorship, respectively (Fig. 1C, E, F). Large decreases in survivorship occurred between 1 and 4 d for all concentrations of spinosad and 4 ppm fipronil.

Comparison of survivorship curves of living (active + incapacitated) with active flies appeared similar for treatments of clothianidin, deltamethrin, and fipronil (compare Fig 1B with 2B, 1D with 2D, 1E with 2E, respectively), but differed for the other three insecticides. The number of active flies increased from 1 to 4 d after feeding on bait containing 400 ppm acetamiprid, which indicated that some flies which had been incapacitated were now active (Fig. 2A). The percent of living flies compared with active flies was 40 and 3%, respectively, 1 d after feeding on bait containing 400 ppm imidacloprid, indicating that most (>90%) living flies were incapacitated (Fig. 1C and 2C, respectively). A large proportion of flies that fed on bait containing 40 and 400 ppm of spinosad were incapacitated, resulting in significantly fewer active than living flies 1-2 d after feeding (compare Fig. 1F with 2F).

More than 80% of flies were knocked down after 1 h on baits containing 400 ppm of acetamiprid, clothianidin, and imidacloprid, with 30% knocked down for fipronil and spinosad (Table 1). Flies exposed to treatments of deltamethrin and control bait were not affected. Compared with the control bait there were significant higher knockdown effects after 1 h for baits containing 40 ppm of acetamiprid (20%), clothianidin (80%), and imidacloprid (63%).

Feeding Assay—5 min

Protein baits containing insecticide had a significant effect on feeding duration ($F = 65.79$; $df = 4, 135$; $P < 0.0001$; Fig. 3). Compared with the control bait flies fed significantly less on baits containing imidacloprid and clothianidin, and fly feeding was not significantly different for baits containing fipronil and spinosad. Bait containing

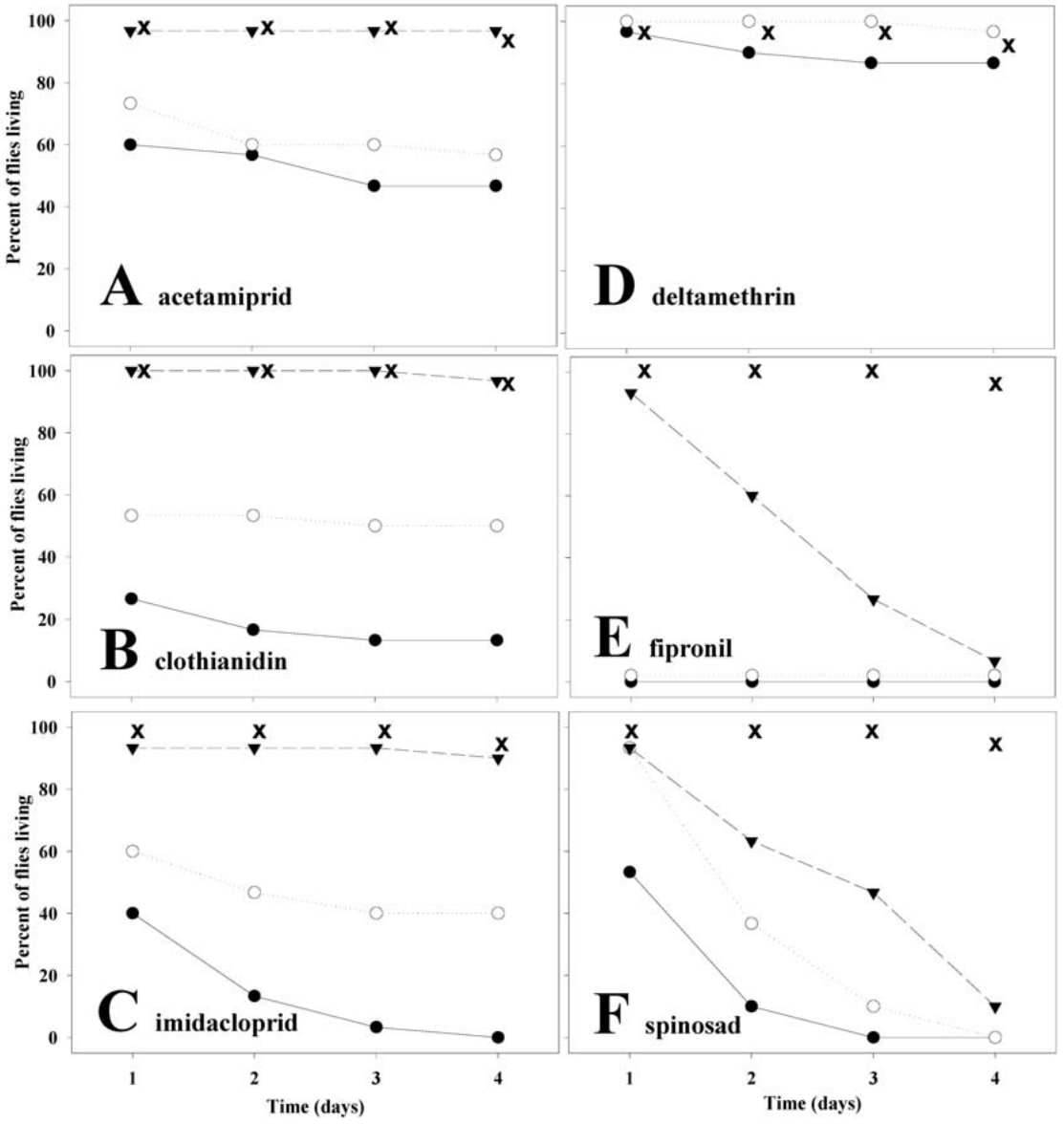

Fig. 1. Percent of flies that were living (active + incapacitated flies) after feeding for 10 s on a droplet of a given insecticide concentration. A) Acetamiprid, B) Clothianidin, C) Imidacloprid, D) Deltamethrin, E) Fipronil, F) Spinosad; (▼ = 4 ppm, ○ = 40 ppm, ● = 400 ppm insecticide; X = control bait without insecticide) (Deltamethrin was not evaluated at 4 ppm.)

imidacloprid was the only treatment that resulted in knockdown after 1 h that was significantly higher than the control (χ^2 with Bonferroni correction, $P = 0.05$; Table 2). Flies that fed on fipronil were dead after one day and flies that fed on spinosad were all dead after four days; and both results were significantly higher than the fly mortality in the control (χ^2 with Bonferroni correction, $P = 0.05$; Table 2).

Exposure—4 h

All insecticide treatments resulted in significantly higher knockdown than the control except spinosad after 3 h ($F = 4.47$; $df = 4, 15$; $P = 0.014$; Table 3). After 24 h, treatments had a significant effect on fly mortality ($F = 3.58$; $df = 4, 15$; $P = 0.031$; Table 3), with all insecticide treatments resulting in significantly higher mortality than the

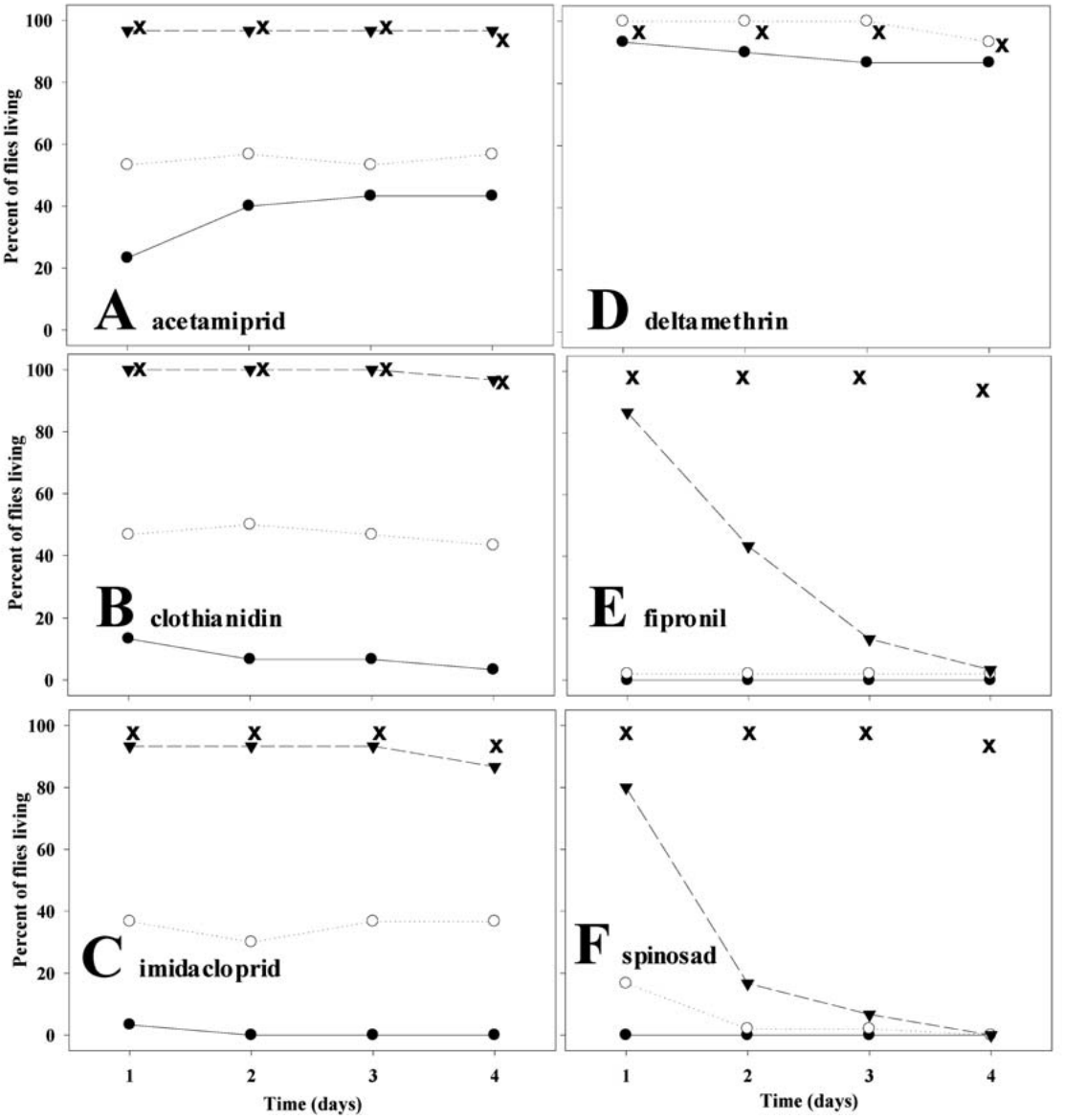

Fig. 2. Percent of flies that were active (with incapacitated flies excluded) after feeding for 10 s on a droplet of a given insecticide concentration. A) Acetamiprid, B) Clothianidin, C) Imidacloprid, D) Deltamethrin, E) Fipronil, F) Spinosad; (▼ = 4 ppm, ○ = 40 ppm, ● = 400 ppm insecticide; X = control bait without insecticide) (Deltamethrin was not evaluated at 4 ppm.)

control except imidacloprid. After 48 h, there were no differences among the treatments including the control (F = 1.12; df = 4, 15; P = 0.385; Table 3).

Survivorship Unlimited Access—Fresh bait

Feeding on fresh bait containing insecticide resulted in significant fly knockdown after 1 h (F = 7.45; df = 5, 54; $P < 0.0001$; Table 4). Significantly more flies were knocked down on treatments of bait containing acetamiprid, clothianidin, and imidacloprid compared with the control or treatments containing fipronil and spinosad. After 24 and 48 h, treatments had a significant effect on fly mortality (F = 14.86; df = 5, 54; $P < 0.0001$; and F = 15.65; df = 5, 54; $P < 0.0001$, respectively). After 24 h, fly mortality was significantly higher on fipronil and spinosad baits compared with the other three insecticide treatments, all of which

TABLE 1. KNOCKDOWN OF FLIES 1 H AFTER FEEDING FOR 10 S ON INSECTICIDAL BAIT.

Treatment	Fly knockdown (%)		
	400 ppm	40 ppm	4 ppm
Acetamiprid	83 a	20 b	0
Clothianidin	96 a	79 a	0
Deltamethrin	0 c	0 b	—
Fipronil	30 b	0 b	0
Imidacloprid	100 a	63 a	3
Spinosad	30 b	0 b	0
Control	0 c	0 b	0
			NS

Values in the same column having the same letter are not significantly different (multiple chi-square tests after Bonferroni corrections; $P = 0.05$).
$n = 600$ flies.

were significantly higher than the control. These relative treatment relationships were the same after 48 h.

Survivorship Unlimited Access—1-d old bait

Treatments of 1-d old bait containing insecticides had a significant effect on fly knockdown ($F = 8.49$; $df = 5, 54$; $P < 0.0001$; Table 4). The highest numbers of flies were knocked down on acetamiprid, followed by imidacloprid, with both significantly higher than the control. The other three insecticides were not different from the control in fly knockdown. After 24 and 48 h, treatments had a significant effect on fly mortality ($F = 8.88$; $df = 5, 54$; $P < 0.0001$; and $F = 9.9$; $df = 5, 54$; $P < 0.0001$, respectively). After 24 h, fly mortality was

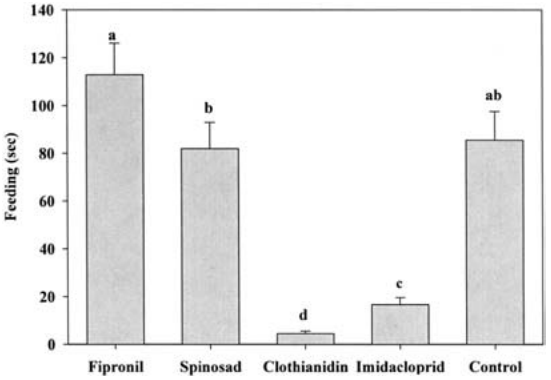

Fig. 3. Duration of fly feeding (mean ± SE) on a bait containing 40 ppm insecticide. Flies were allowed to feed a maximum of 5 min on a 10-µl droplet. The control was bait without insecticide. Vertical bars with the same letter are not significantly different. (Fisher's LSD test with log transformed data). ($F = 65.79$; $df = 4, 135$; $P < 0.0001$).

TABLE 2. FLY MORTALITY AND KNOCKDOWN AFTER 5 MIN EXPOSURE TO BAIT CONTAINING 40 PPM INSECTICIDE.

Treatment	Knockdown (%)	Mortality (%)	
	1 h	1d	4d
Clothianidin	55 ab	15 ab	25 ab
Fipronil	5 ab	100 a	100 a
Imidacloprid	80 a	20 ab	50 ab
Spinosad	5 ab	40 ab	100 a
Control	0 b	0 b	5 b

Values in the same column having the same letter are not significantly different (multiple chi-square tests after Bonferroni corrections; $P < 0.05$).
$n = 100$ flies.

significantly higher on fipronil and spinosad baits compared with the control and the other insecticide baits. After 48 h, baits containing fipronil and spinosad resulted in significantly higher mortality than baits containing either acetamiprid or clothianidin, with the latter two baits resulting in significantly higher mortality than either the control bait or bait containing imidacloprid.

DISCUSSION

Novel compounds were initially evaluated to find replacements for organophosphates and carbamates. Results of several insecticides warrant future field trials to determine the efficacy of different bait spray formulations for controlling *R. mendax*. Compounds differed in their ability to incapacitate and kill flies. Depending on the insecticide chosen for inclusion in protein baits, the modes of action can be predominantly knockdown (acetamiprid, clothianidin, and imidacloprid) or kill (fipronil and spinosad).

TABLE 3. FLY KNOCKDOWN AND MORTALITY AFTER 4 H EXPOSURE TO BAIT CONTAINING 40 PPM INSECTICIDE.

Treatment	Knockdown (%)	Mortality (%)	
	3 h	24 h	48 h[1]
Clothianidin	11.3 ± 1.3 a	13.8 ± 3.8 a	25.0 ± 6.5
Fipronil	8.8 ± 4.3 a	26.3 ± 9.4 a	37.5 ± 14.4
Imidacloprid	5.0 ± 2.0 a	12.5 ± 4.3 ab	23.8 ± 7.2
Spinosad	3.8 ± 1.3 ab	16.3 ± 2.4 a	35.0 ± 7.4
Control	0.0 ± 0.0 b	3.8 ± 2.4 b	16.3 ± 5.9

Values in the same column having the same letter are not significantly different (Fisher's LSD test, $P = 0.05$).
[1]NS, ANOVA, $P > 0.05$.
$n = 400$ flies.

TABLE 4. FLY KNOCKDOWN AND MORTALITY AFTER EXPOSURE TO BLUEBERRY LEAVES CONTAINING BAIT DROPLETS WITH 40 PPM INSECTICIDE.

Experiment	Treatment	Knockdown (%)	Mortality (%)	
		1 h	24 h	48 h
Fresh[1]	Acetamiprid	12.0 ± 4.4 a	42.0 ± 6.9 b	68.0 ± 4.4 b
	Clothianidin	24.0 ± 8.3 a	58.0 ± 8.6 b	78.0 ± 4.6 b
	Fipronil	0.0 ± 0.0 b	80.0 ± 4.2 a	96.0 ± 2.6 a
	Imidacloprid	16.0 ± 4.0 a	44.0 ± 4.9 b	68.0 ± 8.5 b
	Spinosad	0.0 ± 0.0 b	80.0 ± 6.6 a	94.0 ± 3.0 a
	Control	0.0 ± 0.0 b	14.0 ± 2.1 c	30.0 ± 9.1 c
1-d-old[1]	Acetamiprid	16.0 ± 4.0 a	30.0 ± 7.4 b	56.0 ± 10.2 b
	Clothianidin	2.0 ± 2.0 bc	28.0 ± 8.0 b	58.0 ± 9.1 b
	Fipronil	0.0 ± 0.0 c	60.0 ± 6.6 a	86.0 ± 5.2 a
	Imidacloprid	6.0 ± 3.0 b	10.0 ± 4.4 b	30.0 ± 7.4 c
	Spinosad	0.0 ± 0.0 c	68.0 ± 6.8 a	88.0 ± 4.4 a
	Control	0.0 ± 0.0 c	16.0 ± 10.2 b	22.0 ± 10.5 c

For each experiment, values in the same column having the same letter are not significantly different (Fisher's LSD test, $P = 0.05$).

[1] $n = 300$ flies.

Bait sprays containing feeding stimulants (e.g., sucrose) have several advantages to conventional sprays. Lower concentrations of insecticide are needed in bait sprays than conventional sprays because mortality is primarily from oral toxicity, which has lower LC50 thresholds than dermal toxicity, and more insecticide is consumed because of the presence of feeding stimulants (e.g., sucrose) (Hu et al. 2000; Reissig 2003; Barry & Polavarapu 2004). Therefore, baits sprays can be applied at a lower rate of active ingredient per hectare than conventional sprays. The attraction and feeding responses of flies to bait sprays have led to evaluations assessing their potential use as border sprays (Prokopy et al. 2003; Prokopy et al. 2004).

Fly survivorship differed based on concentration and type of insecticide used. As expected, higher concentrations of insecticide resulted in higher mortality of flies. At 400 ppm the shortest lag time between feeding and 100% mortality resulted from bait containing fipronil, followed by bait containing spinosad. The pyrethroid deltamethrin did not result in fly knockdown or mortality that was significant enough to warrant further evaluation on *R. mendax*, which was also the finding of Barry et al. (2004) investigating insecticidal coatings for spheres used in attract and kill of *R. mendax*. The neonicotinoids (acetamiprid, clothianidin, and imidacloprid) resulted in intermediate survivorship, performing better than deltamethrin, but not as well as spinosad or fipronil. Our findings are in agreement with Reissig (2003), who found the LC50 (with flies unable to walk considered dead) of imidacloprid and spinosad for *R. pomonella* to be approximately 11 ppm and between 3-10 ppm, respectively.

Many published insecticide assays involve exposing flies to an insecticide treatment for several days in a small container to determine mortality. These conditions are likely to underestimate the concentration of insecticide needed for fly mortality in the field. The importance of such studies is to determine the suitable type and range of activity for insecticides to be further evaluated. In the current study we used three types of assays to evaluate the effects of insecticides: a variable feeding duration (up to 5 min), a fixed short duration (Feeding Assay—10 s), and a fixed long duration (Survivorship Unlimited Access). Each of these assays has limitations, but taken together supports the findings of the other assays.

Sub-lethal effects of insecticides are known to manifest as a reduction in fecundity, measured indirectly from oviposition punctures by *R. pomonella* (Reissig 2003). In most of the assays in the current study, observations for knockdown occurred 1 h after a fly fed, but flies feeding on the neonicotinoids were often in that state much earlier and later, as evidenced by some flies being unable to feed for the duration of the 10-s trial from becoming incapacitated. Liburd et al (2003) found insecticide-fed flies have lower levels of activity compared with a control. In our study flies that were knocked down often died, but some of the flies in this condition appeared no different than control flies after 1-2 d, apparently recovering from exposure to the insecticide. This finding leads us to suggest that there may be an optimal concentration for consumption to achieve the desired mortality.

Measuring fly mortality in the context of field evaluations of insecticides contained in bait

sprays is one way to determine the effectiveness of knockdown. This would provide a realistic setting in which the effects of natural enemies could be evaluated on flies that are not completely dead, as well as other sub-lethal effects associated with a reduction in oviposition and larval presence. The results of future field trials can determine the effectiveness of bait sprays containing insecticides with different modes of action.

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REFERENCES CITED

AYYAPPATH, R., S. POLAVARAPU, AND M. R. MCGUIRE. 2000. Effectiveness of thiamethoxam-coated spheres against blueberry maggot flies (Diptera: Tephritidae). J. Econ. Entomol. 93: 1473-1479.

BARRY, J. D., AND S. POLAVARAPU. 2004. Feeding and attraction of blueberry maggot flies (Diptera: Tephritidae) to protein baits, ammonium acetate, and sucrose. J. Econ. Entomol. 97: 1269-1277.

BARRY, J. D., S. POLAVARAPU, AND L. A. F. TEIXEIRA. 2004. Evaluation of traps and toxicants in an attract-and-kill system for *Rhagoletis mendax* (Diptera: Tephritidae). J. Econ. Entomol. 97: 2006-2014.

BARRY, J. D., R. I. VARGAS, N. W. MILLER, AND J. G. MORSE. 2003. Feeding and foraging of wild and sterile Mediterranean fruit flies (Diptera: Tephritidae) in the presence of spinosad bait. J. Econ. Entomol. 96: 1405-1411.

BOSTANIAN, N. J., AND G. RACETTE. 2001. Attract and kill, an effective technique to manage apple maggot, *Rhagoletis pomonella* (Diptera: Tephritidae) in high density Quebec apple orchards. Phytoprotection 82: 25-34.

CANADIAN FOOD INSPECTION AGENCY. 1999. Directive D-99-02. Requirements for the import and domestic movement of fresh blueberries and fruit of other hosts of blueberry maggot moving from regulated areas in Canada and the United States to non-regulated areas in Canada. Plant Products Directorate, Plant Health and Production Division, Canadian Food Inspection Agency, Nepean, Ontario.

CORNELIUS, M. L., L. NERGEL, J. J. DUAN, AND R. H. MESSING. 2000. Responses of female oriental fruit flies (Diptera: Tephritidae) to protein and host fruit odors in field cage and open field tests. Environ. Entomol. 29: 14-19.

DOWELL, R. V. 1994. Alternatives to aerial malathion and bait sprays, pp. 49-57 *In* J. G. Morse, R. L. Metcalf, J. R. Carey, and R. V. Dowell [eds.], The Medfly In California: Defining Critical Research. University of California, Center for Exotic Pest Research, Riverside, CA.

DUAN, J. J., AND R. J. PROKOPY. 1992. Visual and odor stimuli influencing effectiveness of sticky spheres for trapping apple maggot flies *Rhagoletis pomonella* (Walsh) (Diptera, Tephritidae). J. Appl. Entomol. 113: 271-279.

DUAN, J. J., AND R. J. PROKOPY. 1993. Toward developing pesticide-treated spheres for controlling apple maggot flies, *Rhagoletis pomonella* (Walsh) (Dipt., Tephritidae): I. Carbohydrates and amino acids as feeding stimulants. J. Appl. Entomol. 115: 176-184.

DUAN, J. J., AND R. J. PROKOPY. 1995a. Development of pesticide-treated spheres for controlling apple maggot flies (Diptera: Tephritidae): Pesticides and residue-extending agents. J. Econ. Entomol. 88: 117-126.

DUAN, J. J., AND R. J. PROKOPY. 1995b. Control of apple maggot flies (Diptera: Tephritidae) with pesticide-treated red spheres. J. Econ. Entomol. 88: 700-707.

FABRE, F., P. RYCKEWAERT, P. F. DUYCK, F. CHIROLEU, AND S. QUILICI. 2003. Comparison of the efficacy of different food attractants and their concentration for melon fly (Diptera: Tephritidae). J. Econ. Entomol. 96: 231-238.

FOOD QUALITY PROTECTION ACT. 1996. Law No. 104-170. U.S. Congressional. Record, vol. 142: 1489-1538.

HU, X. P., J. J. DUAN, AND R. J. PROKOPY. 1998. Effects of sugar/flour spheres coated with paint and insecticide on alighting female *Ceratitis capitata* (Diptera: Tephritidae) flies. Fla. Entomol. 81: 318-325.

HU, X. P., R. J. PROKOPY, AND J. M. CLARK. 2000. Toxicity and residual effectiveness of insecticides on insecticide-treated spheres for controlling females of *Rhagoletis pomonella* (Diptera: Tephritidae). J. Econ. Entomol. 93: 403-411.

KATSOYANNOS, B. I., N. T. PAPADOPOULOS, AND D. STAVRIDIS. 2000. Evaluation of trap types and food attractants for *Rhagoletis cerasi* (Diptera: Tephritidae). J. Econ. Entomol. 93: 1005-1010.

KING, J. R., AND M. K. HENNESSEY. 1996. Spinosad bait for the Caribbean fruit fly (Diptera: Tephritidae). Fla. Entomol. 79: 526-531.

LIBURD, O. E., E. M. FINN, K. L. PETTIT, AND J. C. WISE. 2003. Response of blueberry maggot fly (Diptera: Tephritidae) to imidacloprid-treated spheres and selected insecticides. Can. Entomol. 135: 427-438.

LIBURD, O. E., L. J. GUT, L. L. STELINSKI, M. E. WHALON, M. R. MCGUIRE, J. C. WISE, J. L. WILLETT, X. P. HU, AND R. J. PROKOPY. 1999. Mortality of *Rhagoletis* species encountering pesticide-treated spheres (Diptera: Tephritidae). J. Econ. Entomol. 92: 1151-1156.

MOHAMMAD, A. B., AND M. T. ALINIAZEE. 1989. Malathion bait sprays for control of apple maggot (Diptera: Tephritidae). J. Econ. Entomol. 82: 1716-1721.

MORENO, D. S., AND R. L. MANGAN. 2003. Bait matrix for novel toxicants for use in control of fruit flies (Diptera: Tephritidae), pp. 333-362 *In* G. J. Hallman and C. Schwalbe [eds.], Invasive Arthropods in Agriculture. Science Publishers, INC., Enfield, NH, USA.

PECK, S. L., AND G. T. MCQUATE. 2000. Field tests of environmentally friendly malathion replacements to suppress wild Mediterranean fruit fly (Diptera: Tephritidae) populations. J. Econ. Entomol. 93: 280-289.

PROKOPY, R. J. 1975. Apple maggot control by sticky spheres. J. Econ. Entomol. 68: 197-198.

PROKOPY, R. J., S. E. WRIGHT, J. L. BLACK, X. P. HU, AND M. R. MCGUIRE. 2000a. Attracticidal spheres for controlling apple maggot flies: Commercial-orchard trials. Entomol. Exp. Appl. 97: 293-299.

PROKOPY, R. J., I. JACOME, J. PINERO, L. GUILLEN, F. D. FLEISCHER, X. HU, AND M. ALUJA. 2000b. Post-alighting responses of Mexican fruit flies (Dipt., Tephritidae) to different insecticides in paint on attractive spheres. J. Appl. Entomol. 124: 239-244.

PROKOPY, R. J., N. W. MILLER, J. C. PINERO, J. D. BARRY, L. C. TRAN, L. ORIDE, AND R. I. VARGAS. 2003. Effectiveness of GF-120 fruit fly bait spray applied to border area plants for control of melon flies (Diptera: Tephritidae). J. Econ. Entomol. 96: 1485-1493.

PROKOPY, R. J., N. W. MILLER, J. C. PINERO, L. ORIDE, N. CHANEY, H. REVIS, AND R. I. VARGAS. 2004. How effective is GF-120 fruit fly bait spray applied to border area sorghum plants for control of melon flies (Diptera: Tephritidae). Fla. Entomol. 87: 354-360.

REISSIG, W. H. 2003. Field and laboratory tests of new insecticides against the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). J. Econ. Entomol. 96: 1463-1472.

SAS INSTITUTE. 1999. User's manual, version 8.0. SAS Institute, Cary, NC.

SHARP, J. L., AND D. L. CHAMBERS. 1984. Consumption of carbohydrates, proteins and amino acids by *Anastrepha suspensa* (Diptera: Tephritidae) in the laboratory. Environ. Entomol. 13: 768-773.

STEINER, L. F. 1952. Fruit fly control in Hawaii with poisoned sprays containing protein hydrolysate. J. Econ. Entomol. 45: 838-843.

STELINSKI, L. L., O. E. LIBURD, S. WRIGHT, R. J. PROKOPY, R. BEHLE, AND M. R. MCGUIRE. 2001. Comparison of neonicotinoid insecticides for use with biodegradable and wooden spheres for control of key *Rhagoletis* species (Diptera: Tephritidae). J. Econ. Entomol. 94: 1142-1150.

VARGAS, R. I., S. L. PECK, G. T. MCQUATE, C. G. JACKSON, J. D. STARK, AND J. W. ARMSTRONG. 2001. Potential for areawide integrated management of Mediterranean fruit fly (Diptera: Tephritidae) with a braconid parasitoid and a novel bait spray. J. Econ. Entomol. 94: 817-825.

ECOLOGY OF CRABRONID WASPS FOUND IN TRAP NESTS FROM SPAIN (HYMENOPTERA: SPHECIFORMES)

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ABSTRACT

We report data obtained concerning the occupation of trap nests by xylicolous Crabronidae (*sensu* Melo 1999) in a study carried out in central Spain between 1992 and 1995. In particular, we analyze the data on the occupation of the nests for *Psenulus concolor* (Dahlbom), *Trypoxylon attenuatum* F. Smith, and *Trypoxylon beaumonti* Antropov. All three species use pre-existing cavities of 2-4 mm to establish their nests. The mortality rates varied between 33% and 55%, and of special interest was the variation between the two species of *Trypoxylon* L. and the absence of mortality due to natural enemies in *P. concolor*. In the three species, mortality was similar along the nests, with no increase in the innermost or outermost cells. *Trichrysis cyanea* (L.) was the most abundant natural enemy in the nests analyzed. Sex distribution was not random in any of the species studied: in *P. concolor* and *T. attenuatum*, the males developed in the outermost cells, while in *T. beaumonti* they appeared in the innermost ones. The sex ratio did not deviate from 0.5 in *P. concolor* and *T. attenuaum*, although in *T. beaumonti*, the number of females was significantly higher than that of males.

Key Words: trap-nests, xylicolous, kleptoparasitoids, *Psenulus*, *Trypoxylon*, *Trichrysis*

RESUMEN

Se presentan los datos obtenidos sobre la ocupación de nidos trampa por Crabronidae (*sensu* Melo 1999) xilícolas, en un estudio llevado a cabo en el centro de España, entre 1992 y 1995. Se analizan específicamente los datos de ocupación y contenido de los nidos para *Psenulus concolor* (Dahlbom), *Trypoxylon attenuatum* F. Smith, y *Trypoxylon beaumonti* Antropov. Las tres especies utilizan cavidades preexistentes de 2-4 mm para establecer sus nidos. Las tasas de mortalidad obtenidas varían entre un 33% y un 55%, destacando la variación observada entre las dos especies de *Trypoxylon* L. y la ausencia de mortalidad ocasionada por enemigos naturales en *P. concolor*. En las tres especies la mortalidad es similar a lo largo del nido, no incrementándose en las celdas más externas o internas. *Trichrysis cyanea* (L.) es el enemigo natural más abundante en los nidos analizados. La distribución de sexos no es aleatoria en ninguna de las tres especies estudiadas: en *P. concolor* y *T. attenuatum*, los machos se desarrollan en las celdas más externas, mientras que en *T. beaumonti* aparecen en las celdas más internas del nido. El sex ratio no se aparta de 0.5 en *P. concolor* y *T. attenuaum*, aunque en *T. beaumonti*, el número de hembras es significativamente mayor que el de machos.

Translation provided by the authors.

The Crabronidae include a large number of xylicolous species. These wasps build their nests either in soft core or hollow stems and even in soft pieces of wood. They may excavate their own nests (such that they function as true constructors) or may occupy pre-existing nests or empty holes.

The nests may be linear, with cells located one after the other, or branched; the latter are never found in hollow stems. In both cases, the cells are divided by a septum of mud, resin, or wood particles. According to Krombein (1967) the septa "serve to protect against parasites, parasitoids, and predators; they ensure the nutrition of the larvae and prevent cannibalism; they serve as orientation for the exit of the adult". In some cases, there is also a plug at the end of the gallery that forms a "vestibular cell" in front of the last provisioned cell.

Here we report data obtained in a study with trap-nests on 9 species of crabronids (*sensu* Melo 1999): *Passaloecus gracilis* (Curtis, 1834), *P. singularis* (Dahlbom, 1844), *Pemphredon lethifer* (Suckard, 1837), *Psenulus concolor* (Dahlbom, 1843), *Spilomena troglodites* (Van der Linden, 1829), *Stigmus solskyi* Morawitz, 1864; *Trypoxylon attenuatum* F. Smith, 1851, *T. beaumonti* Antropov, 1991, and *T. minus* Beaumont, 1945. However, we only analyze the data from those species whose samples can be considered representative: *Psenulus concolor*, *Trypoxylon attenuatum* and *T. beaumonti*.

Psenulus Kohl includes about 160 species, of which 10 have been found in Europe. The biology and ecology of the European species have been described by Freeman (1938); Grandi (1961); Janvier (1962, 1975); Danks (1970, 1971a); Jacob-Remacle

(1976, 1985, 1986); and Bonelli (1988), who provided data concerning nest structure, prey, and parasitoids. Lomholdt (1976); Bohart & Menke (1976); and Dolfuss & Bitsch (2001) compiled data published by other authors, among which the references to natural enemies are outstanding.

Trypoxylon L. is a cosmopolitan genus with around 700 species. The presence of 17 species has been recorded in Europe (Antropov 2001). Many works have addressed the biology of the European species, most of them referring to nest structure, prey identification, and parasitoids. Some works are purely descriptive or just compilations, such as those by Hamm & Richards (1930); Maréchal (1936); Freeman (1938); Bristowe (1948); Binaghi (1956); Grandi (1961); Abraham (1982); and Antropov (2001). Danks (1970, 1971a, b); Jacob-Remacle (1976, 1985, 1986, 1987); and Asís et al. (1994) conducted surveys that, unlike previous works, quantified data obtained about nests, prey, natural enemies, and mortality. Of the species addressed in the present work, *T. attenuatum* has been studied by Danks (1970) and Asís et al. (1994), while *T. beaumonti* biology is almost unknown.

Materials and Methods

One thousand and seventeen trap-nests were placed in 19 localities of Burgos, Cuenca, León, Segovia, Soria, Teruel, Valladolid, and Zamora provinces, from central-western Spain, and 931 were collected at the end of the study. The trap-nests, made from stems of *Ailanthus altissima* Swingle (Simaroubaceae) (l = 20-30 cm; d = 2-14 mm) and *Phragmites australis* (Cav.) (Poaceae) (l = 20-30 cm; d = 1-8 mm) (cane) were grouped in sheaves with four stems each. The sheaves represented one of the following models: a) four cane stems of different diameters (≥2 mm); b) two stems of *Ailanthus* and two of cane; c) four stems of cane of 1-2 mm. These sheaves were placed in the field at the beginning of the spring of 1992, 1993, 1994 and 1995, attached to the branches of trees with insulating tape, and were withdrawn in the autumn of each of the above years. Thus, they remained in place for 6-7 months. Once collected, the sheaves were placed in an Iar (model CF 85) refrigerator at 6-8°C. The stems were opened later, any occupation by aculeates was determined, and stems with nests inside were studied.

The data gathered were primarily diameter and length of the built nest, cell numbers and contents, and presence of vestibular cells and septa. The cells were numbered from the exterior to the interior (cell 1 being the outermost one), although chronologically the innermost cell was the one constructed first. The contents of each cell were transferred to glass vials, which were held in a refrigerator (6-8°C) until the following spring, when they were transferred to a Heraeus culture chamber (28°C) to promote the emergence of adults. It was then possible to identify the occupants and some of their parasitoids. The total number of nests studied was 511. Between 15% and 25% of the mature larvae were conserved for a possible later study of the preimaginal stages. This means that the mortalities, calculated as a function of the adults obtained, are slightly overestimated (because mortality affecting immature larvae could not act on mature larvae).

The stems of each class and diameter that were occupied, unoccupied, or abandoned were counted. The calculations regarding occupancy were carried out based on the number of "nesting sites" (approximately double the number of stems) since two nests could have been placed in each of them: one on each side. Possible reoccupation (stems occupied in the first and second generation) was not taken into account in determining global occupation index.

Analyses were limited to the wasps *Psenulus concolor*, *Trypoxylon attenuatum*, and *T. beaumonti* (Crabronidae), and kleptoparasitoid *Trichrysis cyanea* (L., 1758) (Chrysididae). Data refer to individuals of the second generation, because only one collection was made, at the end of the summer. Abandoned nests (i.e., nests occupied during the first generation and abandoned by the adults) were considered to be susceptible to occupation during the second generation.

The sex ratio was calculated as the "number of males/total number of adults obtained". The following abbreviations are employed: M1 = mortality in the egg stage, including the possible absence of oviposition; M2 = mortality of the different larval stages (with the exception of the mature larva); M3 = mortality due to natural enemies; M4 = mortality in the mature larval stage or in metamorphosis giving rise to the adult.

Results

Trap Nest Occupation

The occupation index was 19.34%, slightly higher for the *Ailanthus* stems (19.75%) as compared with those of cane (19.28%) (Table 1).

In cane stems (Table 1), the differences in occupation, as a function of diameter, were significant ($\chi^2_3 = 31.37$; $P < 0{,}0001$), those with a diameter of 3-4 mm being the ones most used. This is because these diameters are better adapted to the size of *Trypoxylon attenuatum* and *T. beaumonti*, the species that established the greatest number of nests (Table 2). In *Ailanthus* stems, a preference as a function of diameter was not observed ($\chi^2_3 = 0.57$; $P = 0.90$) (Table 1).

Trypoxylon was the most abundant genus (272 nests, 72.9%), while *Pemphredon* L. (43 nests) and *Psenulus* (12 nests) had much lower percentages

TABLE 1. STEMS COLLECTED AS A FUNCTION OF DIAMETER AND TYPE (A = ABANDONED, NO = NOT OCCUPIED, O = OCCUPIED, T = TOTAL).

Cane	A (%)	NO (%)	O (%)	T
Total	239 (14.1)	1126 (66.6)	326 (19.3)	1691
1 mm	0 (0.0)	107 (99.1)	1 (0.9)	108
2 mm	30 (5.6)	409 (76.9)	93 (17.5)	532
3 mm	183 (20.0)	537 (58.6)	197 (21.5)	917
4 mm	20 (16.5)	69 (57.0)	32 (26.4)	121
5-8 mm	6 (46.2)	4 (30.8)	3 (23.1)	13
Ailanthus				
total	32 (13.4)	159 (66.8)	47 (19.7)	238
2-4 mm	1 (9.1)	8 (72.7)	2 (18.2)	11
5-7 mm	11 (9.3)	82 (69.5)	25 (21.2)	118
8-10 mm	16 (18.2)	55 (62.5)	17 (19.3)	88
12-14 mm	4 (19.0)	14 (66.7)	3 (14.3)	21

of occupation (11.5% and 3.2%, respectively). The presence of *Spilomena* Shuckard, *Passaloecus* Shuckard, and *Stigmus* Panzer was very reduced, with scarcely 2.9% of the nests among the three genera (Table 2).

Differences among the genera were observed in the number of nests employed by each species in the different types of stems (*Ailanthus*—cane). *Trypoxylon* females established their nests exclusively in pre-existing cavities, its reported presence in *Ailanthus* stems (14 nests, 5.1% of the total found for the genus) probably is anecdotal and undoubtedly due to the occupation of pre-existing galleries in stems excavated by various Apoidea or *Pemphredon*. Although data reported for *Psenulus* and *Passaloecus* are scarce, the data also indicate the use of pre-existing cavities. *Pemphredon* spp. (Table 2) occupied both types of stems (22 nests in *Ailanthus*, 21 in cane). Thus, the females excavated their nests in stems with soft cores, or occupied pre-existing cavities. The data obtained for *Spilomena* and *Stigmus* (Table 2), although also scarce, point towards a preferential use of soft-cored stems.

Wasp Biology

Psenulus concolor. Twelve nests were obtained, 11 in cane stems of 3-4 mm and one in *Ailanthus* (Table 2). The number of cells varied between 2 and 16 ($\bar{x}$ = 9.5 cells). The observed mortality was 48.4%, representing that undergone by mature larvae or 37.7% of those in the process of metamorphosis that gave rise to adults (Table 3). No parasitoids were found attacking this species.

Upon analyzing the mortality in cells as a function of the position they occupied from the exterior, we observed that this was similar in all of them (χ^2_4 = 1.90; NS). The outermost cells did not show a higher mortality index.

The sex ratio obtained was 0.78♂/1♀, not significantly different from 0.5 (binomial test, z =

TABLE 2. CRABRONID NESTS FOUND IN THE DIFFERENT MODELS OF STEMS.

	Cane								
	1 mm	2 mm	3 mm	4 mm	5 mm	5 mm	Total	*Ailanthus*	Total
Total (%)	1 (0.3)	93 (28.5)	197 (60.4)	32 (9.8)	1 (0.3)	2 (0.6)	326	47	373
Passaloecus gracilis	0	2 (100)	0	0	0	0	2	0	2
Passaloecus singularis	0	1 (100)	0	0	0	0	1	0	1
Pemphredon lethifer	0	0	5 (83.3)	1 (16.7)	0	0	6	14	20
Pemphredon sp.	0	0	14 (93.3)	1 (6.7)	0	0	15	8	23
Psenulus concolor	0	0	7 (63.6)	4 (36.4)	0	0	11	1	12
Trypoxylon attenuatum	0	67 (37.8)	95 (53.7)	15 (8.5)	0	0	177	4	181
Trypoxylon beaumonti	0	3 (10.7)	20 (71.4)	3 (10.7)	0	2 (7.1)	28	0	28
Trypoxylon minus	0	0	1 (100)	0	0	0	1	0	1
Trypoxylon sp.	0	15 (28.8)	32 (61.5)	4 (10.7)	1 (1.9)	0	52	10	62
Spilomena troglodytes	0	0	0	0	0	0	0	6	6
Stigmus solskyi	0	0	0	0	0	0	0	2	2
Unknown	1 (3.0)	5 (15.2)	23 (69.7)	4 (12.1)	0	0	33	2	35

TABLE 3. CONTENTS OF NESTS OF *PSENULUS CONCOLOR*, *TRYPOXYLON ATTENUATUM*, AND *T. BEAUMONTI*. M1, M2, M3, M4 = TYPE OF MORTALITY.

	P. concolor (%)	*T. attenuatum* (%)	*T. beaumonti* (%)
Preserved	19	153	32
♀♀	27	209	34
♂♂	21	188	10
Adults	48 (51.6)	397 (66.8)	44 (45.4)
Total mortality	45 (48.4)	197 (33.2)	53 (54.6)
M1	3 (3.2)	44 (7.4)	17 (17.5)
M2	6 (6.5)	26 (4.4)	5 (5.2)
M3	0	14 (2.4)	6 (6.2)
M4	36 (38.7)	113 (19.0)	25 (25.8)
Total cells	112	747	129

0.72; NS). The distribution of males and females inside the nests was not similar ($\chi^2_4 = 15.99$; $P < 0.01$); the sex ratio follows a negative exponential model (sex ratio = -0.396 ln (cell position) + 1.040; $R^2 = 0.825$) ($F_{1,13} = 92.12$; $P < 0,0001$), and hence the males are increasingly less abundant towards the innermost cells.

Trypoxylon. At least three species nested in the stems provided (Table 2): *T. attenuatum*, *T. beaumonti* and *T. minus*, a total of 210 nests being counted. Moreover, 62 nests were detected that could not be assigned to any given species with certainty because the larvae had not developed into adults.

Trypoxylon attenuatum. With 181 nests (177 in cane and 4 in *Ailanthus*) *T. attenuatum* was the most abundant species. Occupation is shown in Table 2 as a function of stem diameter. No preference for any specific diameter was observed within the 2-4 mm range ($\chi^2_2 = 1,82$; NS), although no nests in canes of other diameters were found.

The number of cells per nest varied between 1 and 11 ($\bar{x} = 4.12$). The observed mortality was 33.2%. M4 accounted for the greater part of the mortality (almost 60%), while the incidence of natural enemies was very low, and only 14 cells out of 594 (2.4%) were parasitized (Table 3).

Mortality as a function of the position occupied by the cells from the exterior was found to be quite similar in all of them ($\chi^2_7 = 11.33$; NS). Thus, mortality was not higher in the cells closer to the exterior. Neither were there any significant differences as a function of the number of cells in the nests. Mortality in the nests with few cells was of the same order as in those with more cells ($\chi^2_6 = 8.55$; NS).

The number of males and females obtained was similar (0.9♂/1♀), and did not depart significantly from a sex ratio of 0.5 (binomial test, $z = 1.42$; NS). However, the distribution of males and females within the nests was not the same ($\chi^2_3 = 46.35$; $P < 0.0001$): the males developed in the outermost cells, and the sex ratio follows a negative exponential model (sex ratio= -0.314 ln (cell position) + 0.743; $R^2 = 0.941$) ($F_{1,9} = 76.80$; $P < 0.0001$).

Trypoxylon beaumonti. Twenty-eight nests were obtained, all of them from cane stems (Table 2). Although the data are scarce, there seemed to be a significant preference towards the occupation of galleries of 3-4 mm ($\chi^2_3 = 8.17$; $P < 0.05$). The number of cells varied between 2 and 7 ($\bar{x} = 4.6$).

The observed mortality was 54.6% (Table 3), with six cells attacked by *Trichrysis cyanea*. Mortality varies depending on the position occupied by the cell from the exterior ($\chi^2_3 = 10.48$; $P < 0.01$), being higher for the most exterior cell (80%) and smaller for inner cells (around 40%).

Important differences were seen between the number of males and females obtained (0.29♂/1♀), and the sex ratio departed significantly from 0.5 (binomial test, $z = 3,47$; $P < 0.001$). Sex distribution inside the nests was not similar ($\chi^2_4 = 13.96$; $P < 0.01$), the males being found in the innermost nests and the females being progressively more abundant towards the exterior.

Trichrysis cyanea. This kleptoparasitoid was found in 32 nests, belonging to different species of *Pemphredon* and *Trypoxylon*, and parasitized 44 (37%) of the cells (Table 4). The mean number of cells parasitized per nest was 1.38. Eleven of the nests affected, with two or more cells, had more than 50% of the cells parasitized. No significant differences were observed as a function of the position of the cell. The outermost cells did not exhibit a greater probability of being parasitized than those located more to the interior of the nest ($\chi^2_5 = 9.77$; NS). In three of the 44 cells the kleptoparasitoid did not complete its development, although the larvae managed to become pupae. Of the 41 adults obtained, 23 were male and 18 female, and the sex ratio (1.27 ♂:1♀) was not significantly different from 0.5 (binomial test, $z = 0.63$; NS).

DISCUSSION

In *Psenulus concolor*, the mortality value (48.4%) was higher than that reported in Great

TABLE 4. NESTS AND CELLS OF DIFFERENT HOSTS AFFECTED BY *TRICHRYSIS CYANEA*.

	Nests affected	Total number of cells	Parasitized cells	Nests with 2 or more cells parasitized
Pemphredon sp.	2	10	3	1
Trypoxylon attenuatum	4	23	7	2
Trypoxylon beaumonti	8	29	10	3
Trypoxylon sp.	18	57	24	5
Total	32	119	44	11

Britain for the same species by Danks (1970, 1971b) (29.3% and 26%) ($\chi^2_2 = 10.72$; $P < 0.01$). However, the mean number of adults produced per nest was greater in this study (5 adults/nest) than in those of Danks (1970, 1971b) (4.0-4.2 adults/nest), due to the higher number of cells in the nests analyzed (9.3 in this study as compared with 5.4-5.9 in those of Danks 1970, 1971b). It is striking, nevertheless, that no parasitoids were found attacking this species; all mortality derived from the interruption of development during larval and pupal stages. The same observation was reported and attributed to a paucity of data by Danks (1970).

The mortality rates found for European species of *Trypoxylon* vary between 33.2% (for *T. attenuatum* in this study) and 63.8% (for *T. attenuatum* and *T. figulus* in Danks 1971b). The observed differences are significant ($\chi^2_5 = 144.25$; $P < 0.001$). Mortality in *T. attenuatum* is lower, both in this study (33.2%) and in that of Asís et al. (1994) (44.0%). Furthermore, the values reported by Danks (1971b) for *T. attenuatum* and *T. figulus* (63.8%) and by Jacob Remacle (1986) for *T. clavicerum* and *T. minus* (58.2%) are clearly higher, while the value found for *T. beaumonti* (54.6%) and that given by Danks (1970) for *T. attenuatum* and *T. figulus* (54.6%) do not deviate from the mean values. This shows that the populations of these wasps are subject to important fluctuations, although it seems that *T. attenuatum* in the Iberian Peninsula could have appreciably lower rates than the rest of the species or than other populations of this species present in more northern areas.

It is also possible to observe an important variability in mortality due to different agents, and of special interest is the low mortality attributable to the action of natural enemies in the species analyzed here (2.4-6.4%), whereas the rates found in other works are between 15% and 25%.

In *Trypoxylon*, cases have been described in which the distribution of the individuals of each sex in the nest seems to be irregular (see Cross et al. 1975), although in many cases it follows a defined trend. Thus, in some species the males are found in the innermost cells and the females in the outermost ones (IMOF, inner males outer females) while in other species the inner cells harbor females (IFOM). The IMOF model is the main one among species of the subgenus *Trypargilum*, in which the male remains inside the nest during its provisioning, copulating with the female when she returns to it (Krombein 1967; Medler 1967; Coville & Coville 1980; Coville & Griswold 1983, 1984; Camillo et al. 1993, 1994). In wasps of the subgenus *Trypoxylon*, the model seems to be IFOM (Krombein 1967; Asís et al. 1994; Oku & Nishida 1999). *Trypoxylon attenuatum*, as reported by Asís et al. (1994), follows this model. However, the data obtained for *Trypoxylon beaumonti* seem to reflect the IMOF model. This could be due to a lack of data, although it might also be a reflection of differences in the behavior of this species, an aspect that deserves further attention.

In some species of *Psenulus*, sex ratios that clearly deviate towards a greater production of males have been described (0.86 in Krombein 1967, for *Psenulus pallipes* (Panzer), $z = 5.04$, $P <$ 0,0001; 0.68 in Matthews 2000, for *P. interstitialis* Cameron, $z = 2.19$, $P < 0.05$). Nevertheless, the data obtained by us and those reported by Danks (1970, 1971b) point to a different situation for *P. concolor*, with a sex ratio that does not depart significantly from 0.5. Regarding the species of *Trypoxylon* studied, the sex ratio does not depart significantly from 0.5 in *T. attenuatum*, while it does deviate from that figure, and towards a greater production of females, in *T. beaumonti*. According to the theory of parental inversion proposed by Fisher (1999), in large randomly breeding populations selection will result in equal investment in sons and daughters (hence a sex ratio of 0.5). Since the males are smaller than the females in most solitary wasps and require a lower investment, one would expect a greater production of males than of females (i.e., a sex ratio >0.5). However, in situations such as those that affect certain wasps, with fragmented populations in which there is local competition for mates among brothers, the females will be selected to produce female-biased sex investment ratios (Hamilton 1967; Cowan 1991). This could explain the deviation, towards the production of females, observed in *T. beaumonti*, whereas *T. attenuatum*, with larger and less isolated populations on the Iberian Peninsula, would show sex ratios close to 0.5.

It is also necessary to take into account the influence that can be exerted by the nesting sub-

strate on the sex ratio since different studies have shown that cavities with smaller diameters shift the sex ratio towards the production of males (Krombein 1967; Charnov et al. 1981). The diameters offered do not seem the limit the nesting possibilities of the species of *Trypoxylon* studied because mainly the cavities with a medium-sized diameter were those occupied. However, if the cavities offered are larger or smaller than those usually available in nature, a bias towards a greater production of one of the sexes could arise, as long as there is sexual dimorphism as regards size (Trivers & Hare 1976). In any case, Oku & Nishida (2001) have demonstrated that the use of small samples could lead to mistaken conclusions concerning the sex ratio, so caution should be exercised on drawing conclusions from few data.

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REFERENCES CITED

ABRAHAM, R. 1982. The biology of *Trypoxylon attenuatum* and *T. figulus*. Entomol. Mitt. 114: 137-147.

ASÍS, J. D., J. TORMOS, AND S. F. GAYUBO. 1994. Biological observations on *Trypoxylon attenuatum* and description of its mature larva and of its natural enemy *Trichrysis cyanea* (Hymenoptera: Sphecidae, Chrysididae). J. Kansas Entomol. Soc. 67: 199-207.

ANTROPOV, V. 2001. Tribu des Trypoxylini, pp. 347-384 *In* J. Bitsch [ed.], Faune de France 86—Hyménoptères Sphecidae d'Europe Occidentale—Volumen 3. Fédération Francaise des Sociétes des Sciences Naturelles, Paris. 459 pp.

BOHART, R. M., AND A. S. MENKE. 1976. Sphecid wasps of the world. A generic revisión. University of California Press. Berkeley. 695 pp.

BONELLI, B. 1988. Note sul comportamento di nidificazione di *Psenulus fuscipennis* (Dahlb.), *Tachysphex fulvitarsis erythrogaster* (Costa), *Sphex albisectus* Lep. e Serv. e *Sphex occitanicus* Lep. e Serv. (Hymenoptera-Sphecidae). Boll. Ist. Entomol. "Guido Grandi" (Bologna) 43: 79-88.

BINAGHI, G. 1956. Su di un eccezionale insediamento del nido pedotrofico di *Trypoxylon attenuatum* Smith (Hymenoptera, Sphecidae). Boll. Soc. Entomol. Italiana 86: 8-12.

BRISTOWE, W. S. 1948. Notes on the habits and prey of twenty species of British hunting wasps. Proc. Linn. Soc. London 160: 12-37.

CAMILLO, E., C. A. GARÓFALO, G. MUCCILLO, AND J. C. SERRANO. 1993. Biological observations on *Trypoxylon (Trypargilum) lactitarse* Saussure in southeastern Brazil. Rev. Brasileira Entomol. 37: 769-778.

CAMILLO, E., C. A. GARÓFALO, AND J. C. SERRANO. 1994. Observações sobre a biologia de *Trypoxylon (Trypargilum) rogenhoferi* Kohl (Hymenoptera: Sphecidae). An. Soc. Entomol. Brasil 23: 299-310.

CHARNOV, E. L., R. L. LOS-DEN HARTOGH, W. T. JONES, AND J. VAN DEN ASSEM. 1981. Sex ratio evolution in a variable environment. Nature 289: 27-33.

COVILLE, R. E., AND C. GRISWOLD. 1983. Nesting biology of *Trypoxylon xanthandrum* in Costa Rica with observations on its spider prey (Hymenoptera: Sphecidae; Araneae: Senoculidae). J. Kansas Entomol. Soc. 56: 205-216.

COVILLE, R. E., AND C. GRISWOLD. 1984. Biology of *Trypoxylon (Trypargilum) superbum* (Hymenoptera: Sphecidae), a spider-hunting wasp with extended guarding of the brood by males. J. Kansas Entomol. Soc. 57: 365-376.

COVILLE, R. E., AND P. L. COVILLE. 1980. Nesting biology and male behavior of *Trypoxylon (Trypargilum) tenoctitlan* in Costa Rica (Hymenoptera: Sphecidae). Ann. Entomol. Soc. America 73: 110-119.

COWAN, D. P. 1991. The solitary and presocial vespidae, pp. 33-73 *In* K. G. Ross and R. W. Matthews [eds.], The Social Biology of Wasps. Comstock Publishing Associates, Ithaca, NY. 678 pp.

CROSS, E. A., M. G. STITH, M. G., AND T. R. BAUMAN. 1980. Bionomics of the organ-pipe mud-dauber, *Trypoxylon politum* (Hymenoptera: Sphecidae). Ann. Entomol. Soc. America, 68: 901-916.

DANKS, H. V. 1970. Biology of some stem-nesting aculeate Hymenoptera. Trans. R. Entomol. Soc. London 122: 323-399.

DANKS, H. V. 1971a. Populations and nesting-sites of some aculeate Hymenoptera nesting in Rubus. J. Anim. Ecol. 40: 63-77. Biology of some stem-nesting aculeate Hymenoptera nesting in *Rubus*. J. Anim. Ecol. 40: 63-77.

DANKS, H. V. 1971b. Nest mortality factors in stem-nesting aculeate Hymenoptera. J. Anim. Ecol. 40: 79-82.

DOLLFUSS, H., AND J. BITSCH. 2001. Tribus des Psenini, pp. 14-55 *In* J. Bitsch [ed.], Faune de France 86—Hyménoptères Sphecidae d'Europe Occidentale—Volumen 3. Fédération Francaise des Sociétes des Sciences Naturelles. Paris. 459 pp.

FISHER, R. A. 1999. The genetical theory of natural selection. A Complete Variorum edition. Oxford University Press. New York. 318 pp.

FREEMAN, P. 1938. Notes on the nesting of five species of solitary wasps (Hymenoptera, Sphecoidea). Proc. R. Entomol. Soc. London 13: 1-6.

GRANDI, G. 1961. Studi di un Entomologo sugli Imenotteri superiori. Boll. Ist. Entomol. (Bologna) 25: i-xv, 1-659.

HAMILTON, W. D. 1967. Extraordinary sex ratios. Science 156: 477-488.

HAMM, A. H., AND O. W. RICHARDS. 1930. The biology of the british fossorial wasps of the families Mellinidae, Gorytidae, Philanthidae, Oxybelidae and Trypoxylonidae. Trans. Entomol. Soc. London 78: 95-131.

JACOB-REMACLE, A. 1976. Une opération nichoirs artificiels pour Hyménoptères dans trois jardins de Liège. Bull. Ann. Soc. Entomol. Belgique 112: 219-242.

JACOB-REMACLE, A. 1985. L'occupation plurispécifique des rameaux constituant des nichoirs-pièges pour Hyménoptères Aculéates solitaires et son incidence sur la mortalité des occupants. Bull. Ann. Soc. Roy. Belge Entomol. 121: 396-408.

JACOB-REMACLE, A. 1986. Mortalité de quelques hyménoptéres aculéates nidifiant dans des nichoirs-pièges. Bull. Ann. Soc. Roy. Belge Entomol. 122: 107-118.

JACOB-REMACLE, A. 1987. Influence de l'urbanisation sur les populations d'hyménoptères aculéates xylicoles: étude effectuée à Liège par la méthode des nichoirs-pièges. Natura Mosana 40(1): 3-18.

JANVIER, H. 1962. Recherches sur les Hyménoptères nidifiants aphidivores. IV. Le genre *Diodontus* (Curtis). Ann. Sci. Nat. Zool. Biol. Anim. (Série 12) 4: 489-515.

JANVIER, H. 1975. Nidificación de *Psenulus concolor* (Dahlbom, 1843) (Hymenoptera). Graellsia 29: 117-142.

KROMBEIN, K. V. 1967. Trap nesting wasps and bees. Life histories, nests and associates. Smithsonian Institution, Washington. 570 pp.

LOMHOLDT, O. 1976. The Sphecidae (Hymenoptera) of Fennoscandia and Denmark *in* Fauna Entomologica Scandinavica. 4, part 1:1-224 (1975), part 2:225-452 (1976). Scandinavian Science Press, Klampenborg, Denmark.

MARECHAL, P. 1936. Ethologie des *Trypoxylon* (Hym. Spheg) et observations sur *T. attenuatum* Sm. Bull. Ann. Soc. Entomol. Belgique 76: 373-396.

MATTHEWS, R. W. 2000. Nesting biology of the stem-nesting wasp *Psenulus interstitialis* Cameron (Hymenoptera: Crabronidae: Pemphredoninae) on Magnetic Island, Queensland. Australian J. Entomol. 39: 25-28.

MEDLER, J. T. 1967. Biology of *Trypoxylon* in trap nests in Wisconsin (Hymenoptera: Sphecidae). American Mid. Natur. 78: 344-358.

MELO, G. A. R. 1999. Phylogenetic relationships and classification of the major lineages of Apoidea (Hymenoptera), with emphasis on the crabronid wasps. Sci. Pap. Nat. Hist. Mus., Univ. Kansas 14: 1-55.

OKU, S., AND T. NISHIDA. 1999. Factors affecting female-biased sex ratio in a trap-nesting wasp, *Trypoxylon malaisei*. Res. Popul. Ecol. 41: 169-175.

OKU, S., AND T. NISHIDA. 2001. Presence of single-sex broods under local mate competition in *Trypoxylon malaisei* (Hymenoptera: Sphecidae): adaptation or maladaptation? Ann. Entomol. Soc. America 94: 550-554.

TRIVERS, R. L., AND H. HARE. 1976. Haplodiploidy and the evolution of social insects. Science 191: 249-263.

ENTOMOLOGICAL WEBSITE USAGE PATTERNS

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ABSTRACT

Usage patterns of entomological research websites were examined to assess their current roles as information resources. A 5-year review of logfiles at three Florida entomological research websites indicated that usage has increased since 1999 and that visitors increasingly have taken advantage of Internet search engines to find pages with high information content. Websites provide opportunities for dissemination of information (for example, in sound files or databases) that is difficult to include in traditional refereed publications. Given the rapid growth of website usage, research organizations may wish to consider formal procedures for vetting such information.

Key Words: Internet, world wide web, search engine

RESUMEN

Los patrones de uso de páginas electrónicas para investigación entomológica fueron examinados para determinar sus papeles actuales como recursos de información. Una revisión de un período de 5-años de los registros de archivos de tres páginas electrónicas de investigación entomologica indica que su uso ha aumentado desde 1999 y los visitantes han tomado una ventaja creciente de sistemas de busqúeda de la Red-electrónica para encontrar páginas con un alto contenido de información. Las páginas electrónicas proveen oportunidades para la diseminación de información (por ejemplo, en un archivo de sonido o una base de datos) que son dificiles para incluir en publicaciones tradicionales reguladas. Tomando en cuenta el crecimiento rápido del uso de páginas electrónicas, las organizaciones de investigación tal vez quieren considerar los procedimientos formales para evaluar esta información.

Entomological institutions began establishing websites in the early 1990s to disseminate research and provide membership services (VanDyk 2000). The Florida Entomological Society (FES) was one of the pioneers in this effort, beginning online publication of new issues of the *Florida Entomologist* at the Florida Center for Library Automation (FCLA) in 1994 (Zenger & Walker 2000). Online journals at society and institutional websites have since become important resources for researchers, but few studies have been published on scientific website usage patterns (Davis 2004) or the roles of institutional websites as information resources (Treise et al. 2003; Lederbogen & Trebbe 2003).

In addition to the original online issues of *Florida Entomologist* in .pdf format, FES launched a membership website in 1998 which hosted a search engine for online issues, *FESsite* (Table 1), and began offering an .html version of new articles at BioOne (*www.bioone.org*) in 2002. A search engine for all *Florida Entomologist* issues was initiated at FCLA in 2004. To evaluate usage of current FES website resources in the context of activity at similar websites, a review was conducted of activity between 2000 and 2004 at *FESsite*, the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology website, *CMAVEsite*, and a personal research site at CMAVE, *Perssite*. The usage was compared with activity at the *Florida Entomologist* journal homepage, the American Chemical Society journal server (Davis 2004), and two entomological websites at the University of Florida (UF). The two UF sites were the Entomology Department Newsletter and the highly visited, Featured Creatures site (Table 1), hosted jointly with the Florida Division of Agriculture and Consumer Services (FDACS).

MATERIALS AND METHODS

Website Logfiles

Daily logs from *FESsite* were analyzed from 5-Sep-99 to 31-Dec-04, using Mediahouse or LiveSTATS (Deepmetrix Corp., Gatineu, Quebec, CA). Daily logs from *CMAVEsite* were analyzed from 14-Jan-00 to 24-Dec-04, with Analog (*www.analog.cx*, Cambridge, UK). Additional analyses were conducted on a subset of the CMAVE daily activity in a personal research site, Perssite (Table 1). File-editing records were examined and directory searches were conducted to count the numbers of pages on the sites at the be-

TABLE 1. DESCRIPTIONS AND INTERNET ADDRESSES (URLS) OF FREQUENTLY ACCESSED CMAVE, FES AND UF ENTOMOLOGY WEB PAGES, WITH ASSOCIATED SITE/PAGE NAMES.

Description	URL	Site/Page name
CMAVE website	cmave.usda.ufl.edu	*CMAVEsite*
CMAVE home	cmave.usda.ufl.edu/index.html	*CMAVEhpage*
IFAHI Unit home[1]	cmave.usda.ufl.edu/ifahi/index.html	
Formis database[2]	cmave.usda.ufl.edu/formis/	
CMAVE publ. list	cmave.usda.ufl.edu/publications.html	
Personal website	cmave.usda.ufl.edu/~rmankin	*Perssite*
Personal home	cmave.usda.ufl.edu/~rmankin/index.html	*Pershpage*
Sound library	cmave.usda.ufl.edu/~rmankin/soundlibrary.html	*Perssound*
FES website	flaentsoc.org	*FESsite*
FES home	flaentsoc.org/index.html	*FEShpage*
FlaEnt search[3]	flaentsoc.org/FEASearch.cfm	
FlaEnt home link[4]	flaentsoc.org/fe.html	
FES pests[5]	flaentsoc.org/fespestweblinks2.html	
FES Fla. insects[6]	flaentsoc.org/arthropdiversity/	
UF/FDAC featured creatures	creatures.ifas.ufl.edu/	
UF Entomology newsletter	entnews.ifas.ufl.edu/	

[1]CMAVE Imported Fire Ant and Household Insects Research Unit home.
[2]Bibliography of ant literature.
[3]Link to original search engine for 1993-2004 *Florida Entomologist* online issues.
[4]FES page with information and links to *Florida Entomologist* journal homepage (Fig. 1B).
[5]Links to information about Florida pest insects.
[6]Florida Arthropod Conservation homepage.

ginnings and ends of the analysis periods. The Florida Entomologist home page (*http://www.fcla.edu/FlaEnt/index.htm*) was monitored with Net.Data (IBM, Armonk, NY).

Requests for FES, CMAVE, and personal research website pages were also compared with records of monthly page views of the UF, IFAS Entomology and Nematology Newsletter and the UF, IFAS/FDACS Featured Creatures websites analyzed by LiveSTATS from 1-Jan-02 to 31-Dec-04.

Logfile Analysis Procedures

Logfile software typically provides information about the originators of requests, and the numbers of successful and unsuccessful requests for pages (files with .htm or .html extensions), as well as graphics, .pdf, .wav, or other files embedded in the pages (Srivastava et al. 2000). Unless otherwise specified below, the counts listed in this report refer only to successful requests for (.htm and .html) web pages and not directly to requests for embedded files.

CMAVE and FES website page monthly totals were estimated by summing the counts from all successful views of pages at the website in the 30 days preceding the count. To evaluate the contribution of specific pages to website totals, monthly page views also were counted individually for several frequently accessed FES, CMAVE, and personal research pages (Table 1). The Featured Creatures and Newsletter monthly totals were determined from daily counts.

Monthly totals were counted for page queries initiated by easily identifiable search engines, including Google, MSN, Yahoo, Ask Jeeves, Alta Vista, HotBot, and Lycos. Potential relationships between website page-view rates and search engine query rates were tested with Proc GLM (SAS Institute 1988).

RESULTS

Website and Home Page View Rates

Between 2000 and 2005, the rates of page views of the FES (*FESsite*), CMAVE (*CMAVEsite*), and personal research (*Perssite*) websites increased 2.8-, 6.7-, and 9.5-fold, respectively (Fig. 1A), or 0.6-, 1.3-, and 1.9-fold per year, respectively. The greatest rate of increase was seen at the Featured Creatures website which increased 6.3-fold between January 2002 and December 2004, or 3.1-fold per year. The activity at FES and personal research website pages in 2003-2004 was similar in magnitude to the 3316 monthly queries of the American Chemical Society Server by chemists at Cornell University in 2002-03 (Davis 2004). Apart from the homepage, the *Florida Entomologist* search engine was the most frequently viewed page at *FESsite*. The insect sound library (Leslie 2002) was the most frequently viewed page at *CMAVEsite*. These two pages are considered in greater detail below.

The general trend of increasing rates of page views was modulated by a yearly cycle. As expected with smaller audiences at holidays, the

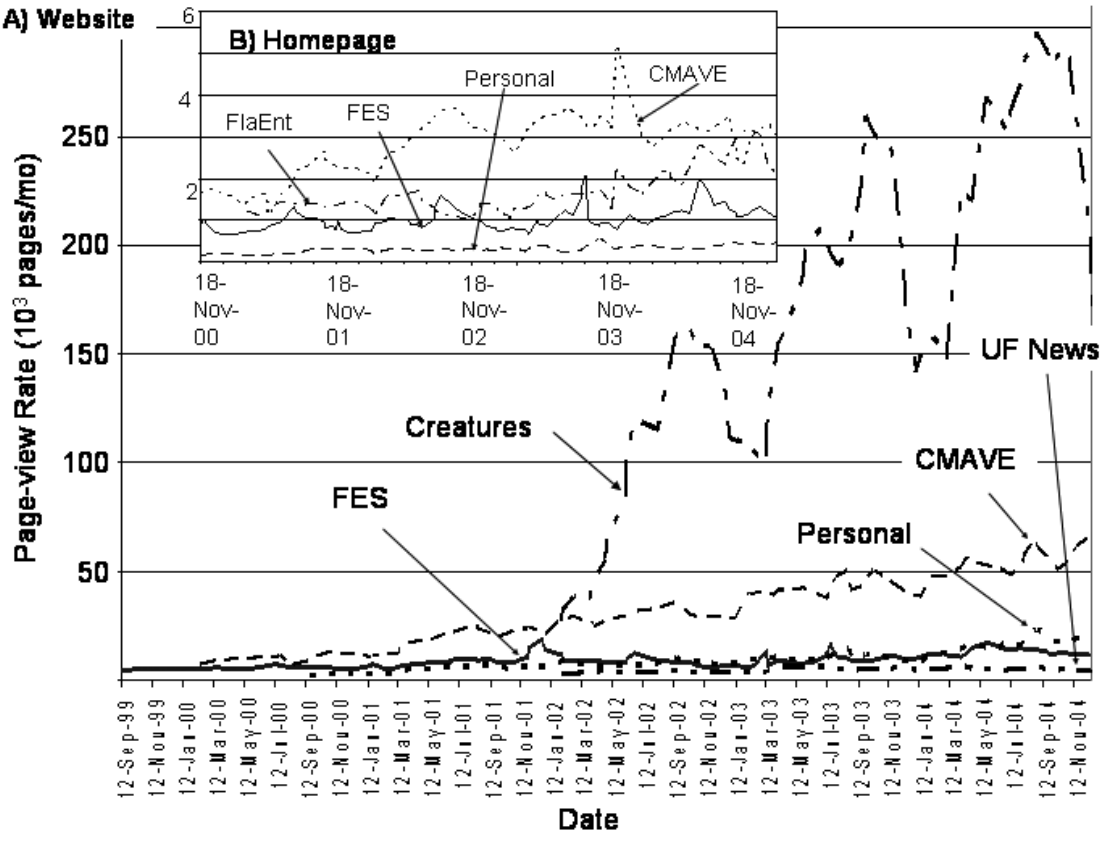

Fig. 1. A) Numbers of Featured Creatures (dot-dashed line), CMAVE (dotted line), FES (solid line), UF Entomology newsletter (dot-dot-dashed line), and Personal website (dashed line) pages viewed monthly between September 1999 and December 2004 (page addresses in Table 1); B) Numbers of CMAVE, FES, Personal, and FlaEnt journal (dot-dashed line) homepages viewed per month between September 1999 and December 2004 (page addresses in Table 1).

view rates at all of the entomological web sites decreased briefly each year in December and January. Increased rates of page views were observed at *FESsite* in each year in June and July, probably due to activities involving the annual meeting. Increased rates of viewing at the Featured Creatures website occurred in August and September, near the beginning of the elementary and secondary school year.

Part of the increased activity between 2000 and 2005 at the FES and CMAVE websites could have been due to an increase in the numbers of web pages at each site, but the percentage increase in page-view rates exceeded the percent-

TABLE 2. REGRESSION EQUATION STATISTICS FOR RELATIONSHIPS BETWEEN PAGE-VIEW RATES AND SEARCH ENGINE QUERY RATES, LISTED BY DECREASING r^2.

Name	F[1]	r^2	Root MSE[2]
Perssite	161.06	0.797	2586.48
FESsite	143.13	0.777	1722.06
CMAVEsite	131.37	0.762	8032.98
Perssound	60.89	0.596	672.64
Pershpage	48.24	0.541	68.14
FEShpage	23.10	0.360	275.28
CMAVEhpage	13.42	0.247	686.24

[1]df = 1,41, $P < 0.001$ for all variables except for *CMAVEhpage*, with $P = 0.007$.
[2]Mean Square Error.

TABLE 3. REGRESSION COEFFICIENT ESTIMATES AND STANDARD ERRORS (SE) FOR RELATIONSHIPS BETWEEN PAGE-VIEW RATES AND SEARCH ENGINE QUERY RATES.

Name (unit)	Estimate	SE	*P*
$A_{CMAVEsite}$ (pages)	13550.130	2299.41	<0.0001
$B_{CMAVEsite}$ (pages/query)	9.750	0.85	<0.0001
$A_{CMAVEhpage}$ (pages)	2202.080	196.43	<0.001
$B_{CMAVEhpage}$ (pages/query)	0.267	0.073	0.007
$A_{FESsite}$ (pages)	1497.300	626.68	0.0216
$B_{FESsite}$ (pages/query)	6.700	0.56	<0.001
$A_{FEShpage}$ (pages)	589.100	100.10	<0.0001
$B_{FEShpage}$ (pages/query)	0.430	0.09	<0.0001
$A_{Perssite}$ (pages)	2141.320	722.57	0.0050
$B_{Perssite}$ (pages/query)	12.830	1.01	<0.0001
$A_{Perssound}$ (pages)	590.070	187.92	0.0031
$B_{Perssound}$ (pages/query)	2.050	0.26	<0.0001
$A_{Pershpage}$ (pages)	203.070	19.04	<0.0001
$B_{Pershpage}$ (pages/query)	0.180	0.03	<0.0001

age increase in web pages. The numbers of web pages increased only from 136 to 619 (455%) at *CMAVEsite*, 85 to 188 (221%) at *FESsite*, and 32 to 84 (262%) at *Perssite*.

In contrast with the website activity, there was less evidence of a trend in the rates of views of FES, CMAVE, and personal research homepages (Fig. 1B). However, there was an increased rate of

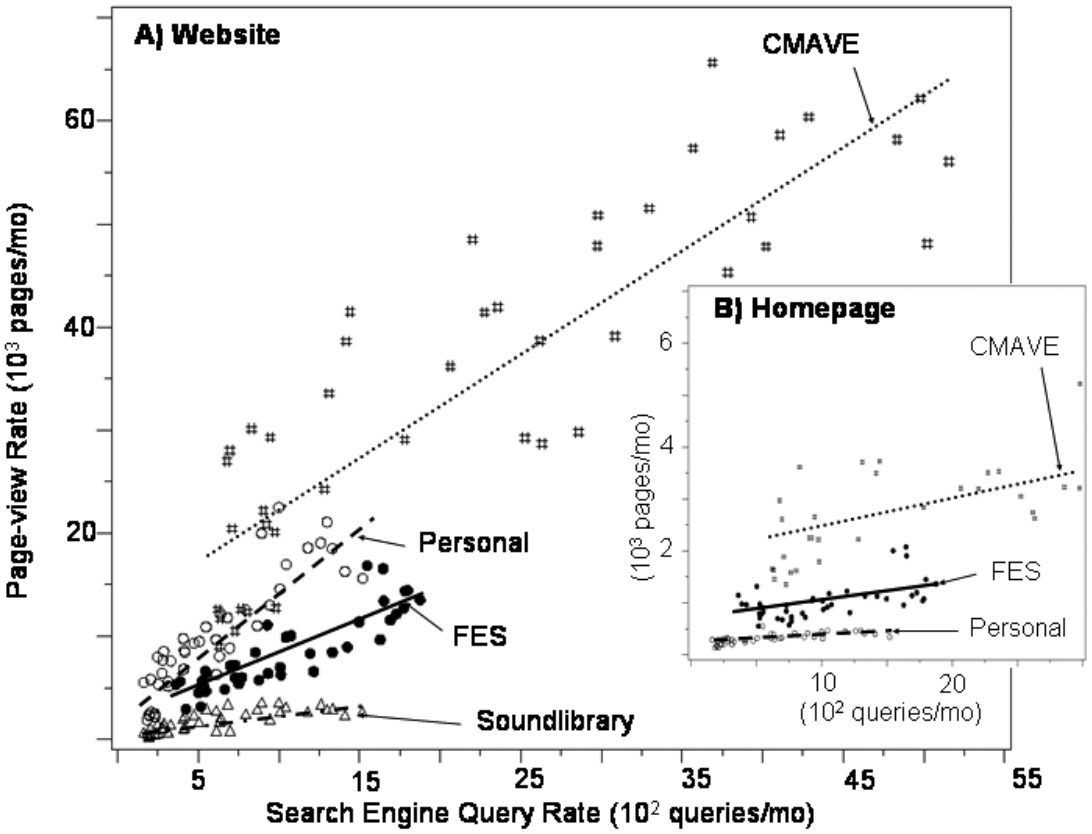

Fig. 2. Comparison of A) website and B) homepage view rates with search engine query rates for CMAVE (hashes, dotted lines), Personal (open circles, dashed lines), FES (dots, solid lines), and Insect sound library (triangles, dot-dashed line), websites.

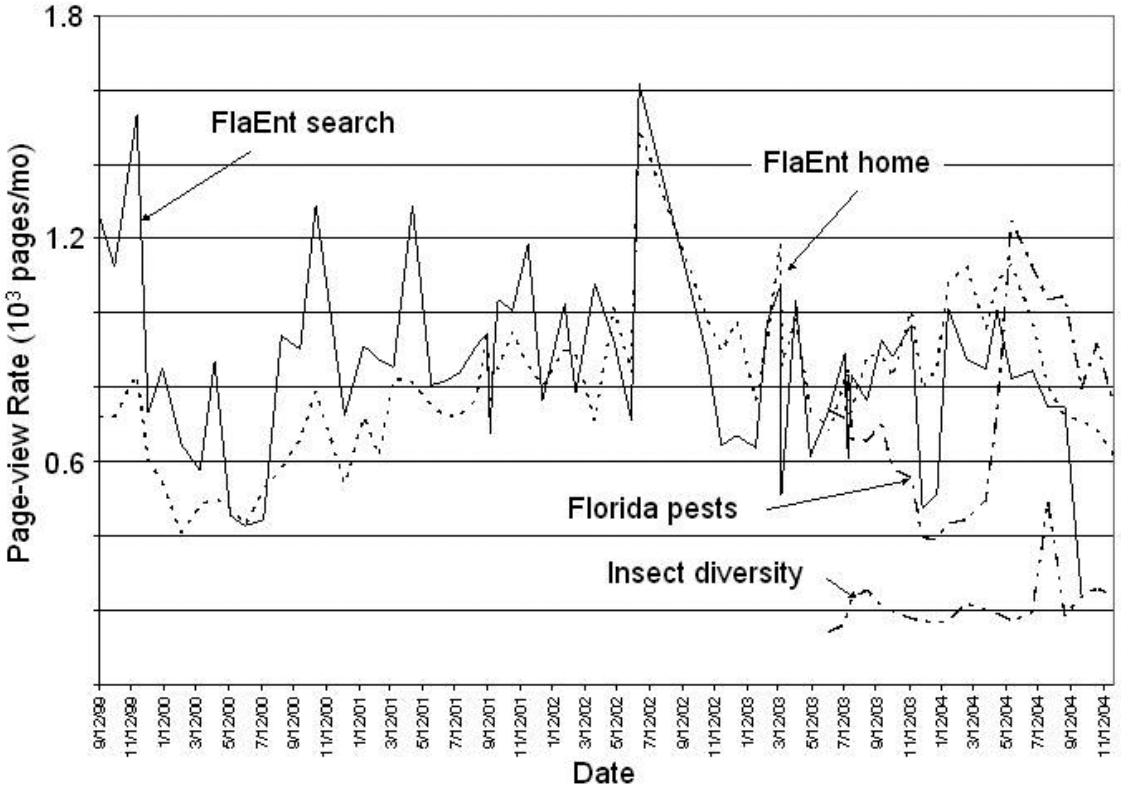

Fig. 3. Rates of viewing of frequently accessed pages at *FESsite*: *Florida Entomologist* text-search engine (solid line), *Florida Entomologist* information page (dotted line), Florida pest control methods (dot-dashed line), insect diversity (dot-dot-dashed line).

viewing of the *Florida Entomologist* journal homepage during 2004 (Fig. 1B) similar to the website trend in Fig. 1A. The difference between the usage patterns for *CMAVEsite*, *FESsite*, *Perssite*, and the corresponding homepages suggests that the behavior of visitors at those three sites (but not at the journal) may have changed over time. If a typical user enters a website through the homepage and then visits five or fewer pages altogether (Cooper 2001), about 15% or more of the pages viewed would be expected to be homepages. Instead, the percentage of homepage views at the CMAVE, FES, and personal websites had declined below 8% by August 2004. Perhaps, experienced visitors were bookmarking pages of interest in one session and then returning to the bookmarked pages directly rather than through the homepage. Alternatively, visitors may have accessed multiple pages directly from a search engine, in which case the rates of website page views might be reflected in search-engine query rates.

Comparisons of Page-Views and Search Engine Queries

The hypothesis that the rates of viewing of pages at CMAVE, FES, and personal research websites between 2000 and 2005 were proportional to search engine query rates was tested under the model:

$$\text{PageViewRate} = A + B\ \text{QueryRate},$$

where PageViewRate is the number of page views per month at a specified website and QueryRate is the number of search engine requests per month for pages at that site. The regressions for the CMAVE, FES, and personal websites, the corresponding home pages, and the insect sound library page are compared in Table 2. The regression coefficients are listed in Table 3 and the relationships are graphed in Fig. 2. The coefficients of determination for website page-view rates and search engine query rates were larger than for the home pages, but all of the regressions in Table 2 were statistically significant.

The slopes of the regression equations for *CMAVEsite*, *FESsite* and *Perssite* (Table 3, Fig. 2A) were all greater than 6 views per query, while the slopes of the regression equations for the corresponding home pages (Fig. 2B) were all less than 0.5 views per query. The large difference in the slopes of the website and homepage page-

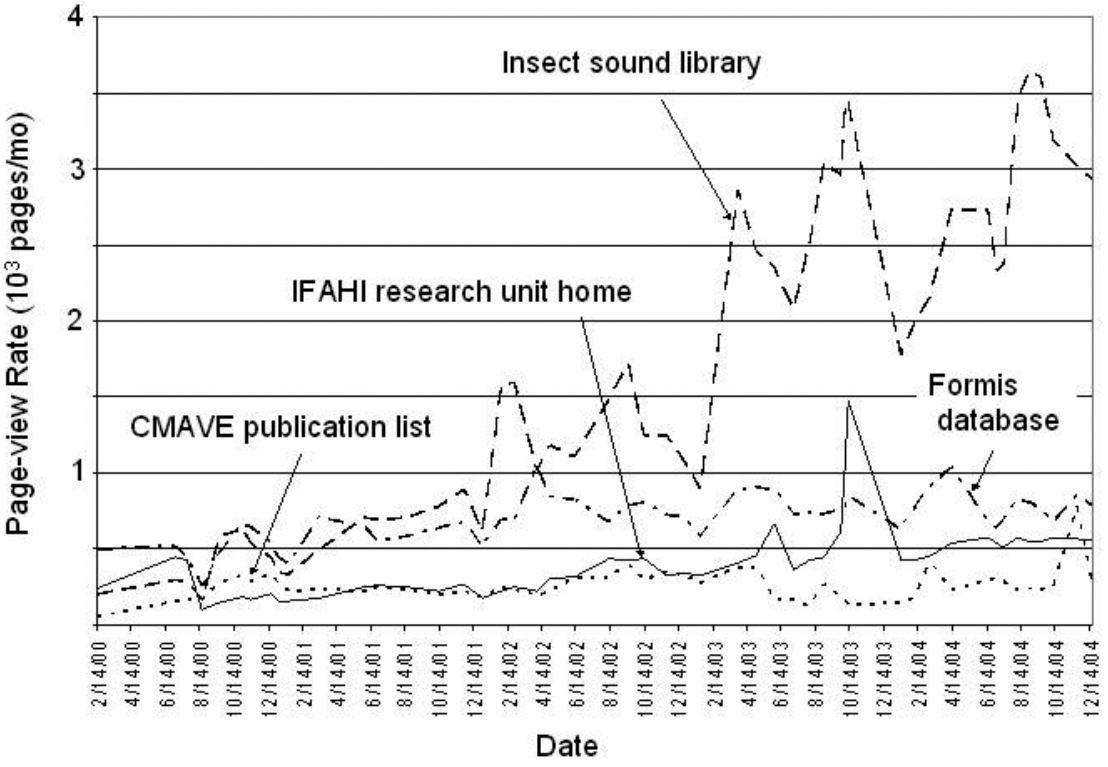

Fig. 4. Rates of viewing of frequently accessed pages at *CMAVEsite*: Insect sound library (dashed line), IFAHI research unit home (solid line), Formis database of ant literature (dot-dashed line), CMAVE publications list (dotted line).

view-rate regressions suggests that many of the viewers had queries in search for specific information present at the site rather than for general information about the hosting institution. Such information most likely would be present in high-content pages rather than homepages. An analysis of frequently viewed pages was conducted to determine whether they were characterized by high levels of information content.

Analysis of Frequently Viewed Pages

Page-view rates for the four most frequently viewed pages other than the website homepages in 2004 are shown for FES in Fig. 3 and CMAVE in Fig. 4. It should be noted that the *Florida Entomologist* search engine page was replaced with an improved version at the FCLA website, which reduced the rate of website page views (Fig. 1B) after September 2004. The introductory page to the *Florida Entomologist* has been viewed frequently since the FES website began, and two pages initiated in 2003 that contain information about Florida pests and Florida arthropod ecology have been viewed at increasing rates in 2004. These, together with the annual meeting program and abstract pages, contain a large fraction of the scientific information available at *FESsite*.

The most frequently accessed pages at *CMAVEsite* other than the homepage in 2004 were the insect sound library, the Formis database, the IFAHI Research Unit home page, and the CMAVE publication list (Fig. 4). The sound library contained information that is not easy to include in a traditional refereed publication, although increasing numbers of journals allow for online posting as supporting online material. The Formis database is a popular source of bibliographic information on ant literature. The CMAVE website has a larger number of high-content pages than *FESsite*, which possibly contributed to its higher rate of page-views in 2004 (Fig. 1A).

Discussion

The patterns described above suggest that increased usage of Internet search engines has se-

lectively increased the rates of viewing of specific FES and CMAVE web pages. Overall, page-view rates have increased, but the greatest increase has occurred for sites and pages with high information content. Search engines typically assign high visibility to research pages from scientific institutions (Jepsen et al. 2004). This has enabled entomological databases, associated software (Byers 2002), and sound files to become important, easily accessible research tools along with the refereed literature. The popularity of the Featured Creatures website may also have benefited from its high ranking in search engines.

The trend of increasingly data-rich scientific websites is not confined to entomology. Research in bioinformatics (Eiden 2004), biodiversity (Maddison & Schulz 2004) and astrophysics (Kurtz et al. 2005), for example, has become highly dependent on digital libraries and online databases. The rapid growth of information indexed by Internet search engines has enabled researchers to modify their search strategies and accelerate their rate of gathering information (Davis 2004). It has also enabled them to disseminate information faster to wider audiences. The improved visibility of Internet-accessible research has resulted already in increased relative effect of open-access research articles published online (Antelman 2004). If such trends continue unabated, researchers could benefit if institutions developed new procedures to vet website content, perhaps similar to the peer review process of scholarly journals. One of the challenges to such development is that web content is frequently updated and, unlike a journal article, would need to be revetted after a major change.

Acknowledgments

Tom Fasulo (University of Florida) provided LiveSTATS records for monthly page views of the Featured Creatures and Entomology Department Newsletter websites. Tom Walker (University of Florida), and Pat Greany, Paul Shirk, Eric Daniels, and Dianne Underwood (CMAVE) have provided assistance and advice in the development of this manuscript.

References Cited

Antelman, K. 2004. Do open-access articles have a greater research impact? College and Research Libraries 65: 372-382.

Byers, J. A. 2002. Internet programs for drawing moth pheromone analogs and searching literature database. J. Chem. Ecol. 28: 807-817.

Cooper, M. D. 2001. Usage patterns of a web-based library catalog. J. Am. Soc. Information Sci. Tech. 52: 137-148.

Davis, P. M. 2004. Information seeking behavior of chemists: A transaction log analysis of referral URLs. J. Am. Soc. Information Sci. Tech. 55: 326-332.

Eiden, L. E. 2004. A two-way bioinformatic street. Science 306: 1437.

Jepsen, E. T., P. Selden, P. Ingwersen, and L. Bjorneböorn. 2004. Characteristics of scientific web publications: preliminary data gathering and analysis. J. Am. Soc. Information Sci. Tech. 55: 1239-1249.

Kurtz, J. J., G. Eichorn, A. Accomazzi, C. Grant, M. Demeitner, and S. S. Murray. 2005. Worldwide use and impact of the NASA Astrophysics data system digital library. J. Am. Soc. Information Sci. Tech. 56: 36-45.

Lederbogen, U., and J. Trebbe. 2003. Promoting science on the web. Science Comm. 24: 333-352.

Leslie, M. 2002. Catch a buzz. Science 297: 743.

Maddison, D. R., and K.-S. Schulz (Ed.). 2004. The tree of life web project. *http://tolweb.org*.

SAS Institute. 1988. SAS/STAT user's guide, release 6.03 edition, Cary, NC.

Srivastava, J., R. Cooley, M. Deshpande, and P.-N. Tan. 2000. Web usage mining: discovery and applications of usage patterns from web data. SIGKDD Explorations 1: 12-23.

Treise, D., K. Walsh-Childers, M. F. Weigold, and M. Friedman. 2003. Cultivating the science internet audience. Science Comm. 24: 309-332.

Vandyk, J. K. 2000. Impact of the internet on extension entomology. Annu. Rev. Entomol. 45: 795-802.

Zenger, J. T., and T. J. Walker. 2000. Impact of the internet on entomology teaching and research. Annu. Rev. Entomol. 45: 747-767.

STRIDULATION OF *GRYLLOTALPA AFRICANA* (ORTHOPTERA: GRYLLOTALPIDAE) ON TURF GRASS IN SOUTH AFRICA

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Abstract

During spring to autumn, *Gryllotalpa africana* males stridulate and produce phonotactic calling songs from specially constructed acoustical burrows. Songs start just after dusk and continue for several hours. The characteristics of the trilling song and sound pressure levels produced were investigated by near field digital recordings made during autumn 2002 and spring 2002 with soil temperatures noted by measuring sound pressures beyond the near field with a sound level meter in spring 2002, respectively. The carrier frequency (2.161-2.477 kHz) and syllable duration (7.340-12.078 ms) of calls showed no significant relationship with soil temperature and no significant differences between autumn and spring with soil temperature constant. Syllable period (10.455-17.221 ms) and inter syllable interval (1.912-9.607 ms) were significantly negatively correlated with soil temperature, and with the latter constant, significantly longer in spring than in autumn. The syllable repetition rate (0.058-0.096 syllables/ms) and duty cycle (43.31-81.72%) showed a significant positive relationship with soil temperature and significant decrease in values with soil temperature constant in spring relative to autumn. Sound pressure levels (re. 20 µPa) at 200 mm from the burrow varied from 77.6 to 89.8 dB.

Key Words: male song characters, seasonal variance, soil temperature, sound pressure level, turf grass

Resumen

Desde la primavera hasta el otoño, los machos de *Gryllotalpa africana* vibran (producen canciones de llamado fonotacticos) de madrigueras acústicas construidas especialmente. Las canciones empiezan un poco después del atardecer y continuan por varias horas. Las características de las canciones vibradas y los niveles de presión del sonido producido fueron investigados por medio de grabaciones digitales en un campo cercano durante el otoño de 2002 y la primavera de 2002 (con la temperatura del suelo anotada) por medio de la medida de la presión de los sonidos (mas alla del campo cercano) con un medidor de nivel de sonido en la primavera de 2002, respectivamente. La frecuencia aportada (2.161-2.477 kHz) y la duración de la sílaba (7.340-12.078 ms) de las llamadas no mostraron una relación significativa con la temperatura del suelo y ningún diferencia significativa entre el otoño y la primavera (con la temperatura del suelo constante). El período de sílaba (10.455-17.221 ms) y el intervalo entre las sílabas (1.912-9.607 ms) fueron negativamente significativas correlacionadas con la temperatura del suelo, y con la constante última, significativamente mas largo en la primavera que en el otoño. La tasa de repetición de sílaba (0.058-0.096 sílabas/ms) y el ciclo obligatorio (43.31-81.72%) mostraron una relación positiva significativa con la temperatura del suelo y una diminución significativa en valores (con la temperatura del suelo constante) en la primavera (en relativa al otoño). Los niveles de la presión del sonido (re. 20 µPa) a 200 mm de la madriguera varian de 77.6 a 89.8 dB.

Numerous insect species produce stereotyped acoustic signals that are important in intraspecific communication (Kavanagh 1987). In most species that communicate by sound, the male's calling song, which appears to attract conspecific females, is the most obvious and imperative component of the repertoire (Kavanagh 1987).

Male African mole crickets differ morphologically from females by having a pair of large cells (anterior of which is the harp) on each forewing, known as the stridulatory area (Townsend 1983) (Figs. 1 and 2). Males usually stridulate at night, using the entrance of borrows as sound amplifiers (De Villiers 1985). Singing position of *Gryllotalpa* sp. appears to be very similar, although acoustic burrows may have two (*G. vineae*, *G. gryllotalpa*, and *G. africana*) (Bennet-Clarke 1970a; Brandenburg et al., 2002) to four horn-shaped openings (*G. australis*) (Kavanagh & Young 1989). The division between openings may collapse over time, producing fewer openings (Bennet-Clarke 1970a & Kavanagh and Young 1989).

Variation between temporally segregated songs of chirping and trilling mole crickets may

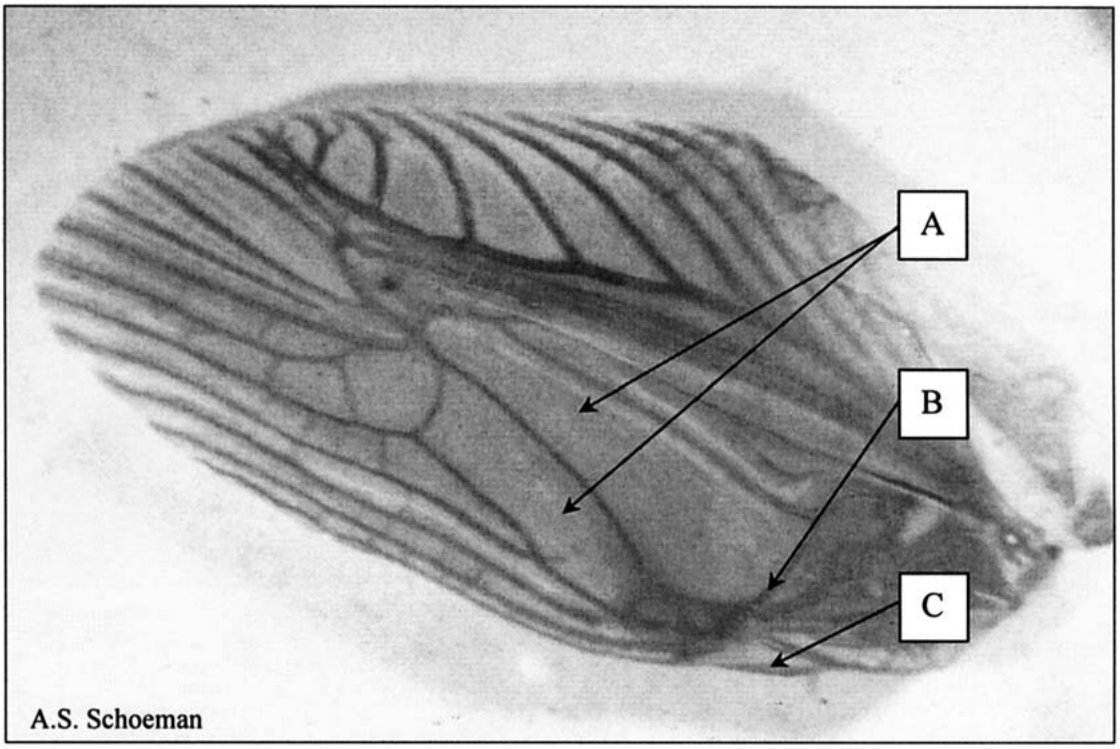

Fig. 1. Ventral view of right male tegmen, showing stridulatory area. A = Stridulatory area, B = File (pars stridens) and C = Scraper (plectrum).

be caused by environmental factor dependence. Chirp rate and syllable or pulse repetition rate in crickets and mole crickets increase linearly with soil temperature over an intermediate temperature range (Bennet-Clark 1970a; Bennet-Clarke 1989; Kavanagh & Young 1989; Doherty & Callos 1991; Ciceran et al. 1994; Hill 1998, 2000). Inter syllable interval is usually negatively correlated with temperature in the Gryllotalpinae and carrier frequency appears to be temperature independent in mole crickets (Bennet-Clark 1989). In the Oecanthinae (Gryllidae), however, the carrier frequency is positively correlated to temperature, but with a smaller slope than for syllable rate (Bennet-Clarke 1989). Walker (1962) also reported carrier frequency to be a regression function of air temperature at low and moderate temperatures for crickets in three genera and three subfamilies. Another potential factor contributing to variation may be physiological, such as size, condition etc. In the Gryllidae, song structure does not, however, appear to vary with male mass or age (Souroukis et al. 1992; Ciceran et al. 1994). In trilling *Gryllotalpa*, the song differences appear to be of fundamental frequency (Bennet-Clark 1970a), while in gryllids, the interval between syllables may be more important (Walker 1962). Male song characteristics in mole crickets are species specific (Bennet-Clark 1970a; Bennet-Clark 1970b; Otte & Alexander 1983; Nickle & Castner 1984; Kavanagh & Young 1989; Walker & Figg 1990; Broza et al. 1998) and provide a key to determine the validity of reports of *G. africana* occurrence.

Sound pressure levels measured just beyond the near field (15-20 cm in line with the burrow, re. 20 µPa) may vary from 65 to 97 dB between trilling mole cricket species, with highest intraspecific sound pressure level variation of 67 to 91 dB (Ulagaraj 1976; Forrest 1983; Bennet-Clarke 1987; Kavanagh & Young 1989; Walker & Forrest 1989). Song intensity of trilling species is positively correlated to male size and usually to temperature, rainfall, and soil moisture (Bennet-Clarke 1970a; Ulagaraj 1976; Forrest 1980; Forrest 1983; Forrest 1991).

Some song characteristics reported for the African mole cricket include a phonotaxis study by Kim (1993) in Hwaseong-gun, Kyonggi-do Korea, who found intensities of calling songs vary between 77 and 80 dB at 150 mm above the openings of calling chambers. The study of Kim (1993) probably does not refer to the "true" *G. africana*. Other song characters of *G. africana* are based on four recordings (Townsend 1983) and vary between reports (Nickle & Castner 1984). Calling

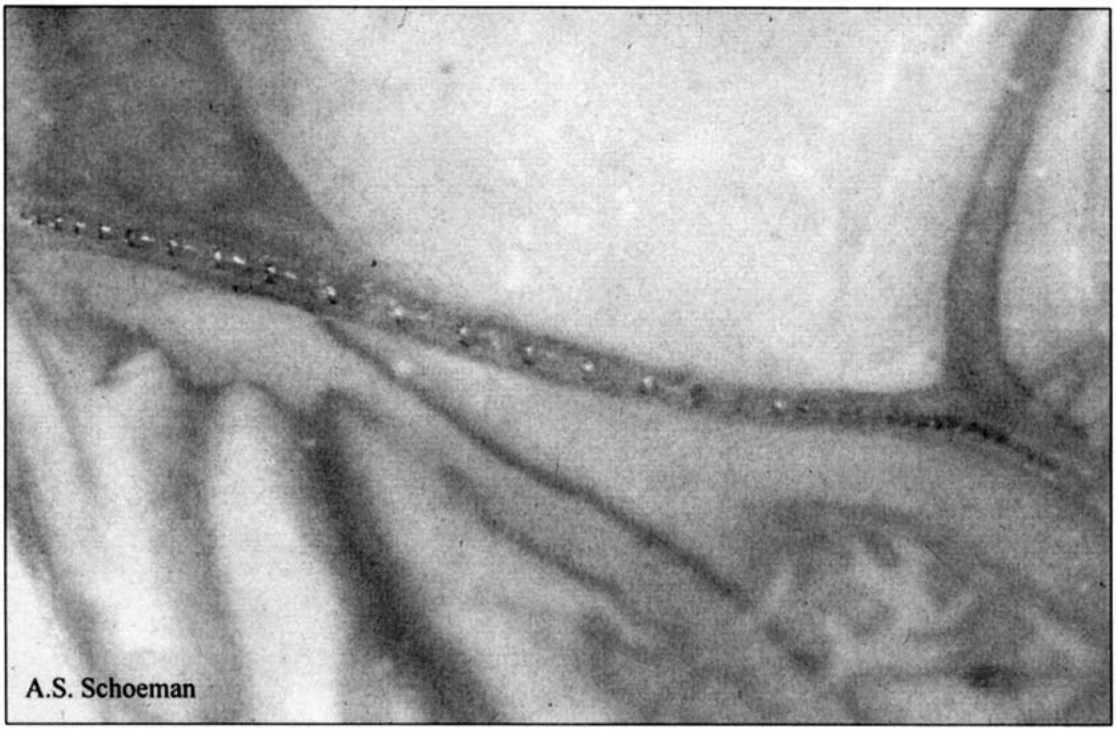

Fig. 2. Ventral view of male tegmen, showing stridulatory teeth arrangement on the file or pars stridens of *G. africana*.

song intensities of *G. africana* from Africa have not been measured.

Materials and Methods

Field recordings (n = 20) of the calling song of *G. africana* males chosen at random but not overlapping were made in a kikuyu grass area of approximately 300 m^2 between and surrounding the putting green and green no. 18 at the Pretoria Country Club from March 2002 to April 2002. Soil temperatures were measured at a vertical depth of 100 mm in the soil profile immediately after recordings were made. Recordings were made between 19h30 and 21h15, local time (GMT + 2 hours). Due to the relative homogeneity including irrigation program, turf grass and soil of the experimental area and relatively short temporal measurement period, soil moisture was assumed to be constant. During October 2002 and November 2002, 20 stridulating males were recorded according to a similar methodology, but at a nearby site comprising a kikuyu grass area (300 m^2) between and surrounding the chipping and bowling green at Pretoria Country Club with a similar irrigation program than the previous site. Recordings between and within the two periods were assumed to be of different males, as no recording sites overlapped. The calls were recorded with a Nomad DAP-3201 digital recorder (Creative Technology Ltd.), with the self-contained microphone held 50 mm from the burrow opening, longitudinal to the long axis of the burrow. Recording distance was within the near field, or distance covered by one wavelength at the carrier frequency of this call (s/2300 cycles × 343 m/s at 20°C = 149.13 mm) (Hill 2000).

All the recordings were analyzed with the computer software program "Canary" V1.2.4 (Cornell Laboratory of Ornithology 1998). A power spectrum (Fig. 3) and oscillogram (Fig. 4) were used to measure three different call characteristics for nine syllables (three successive syllables randomly selected at the beginning, middle and end of each recording, respectively) per recording: Carrier frequency (Fig. 3), syllable duration (Fig. 4) and syllable period (Fig. 4). The inter syllable interval (syllable period – syllable duration), mean syllable repetition rate (inverse of syllable period), and duty cycle ((syllable duration/syllable period) × 100) were calculated from the measured parameters.

The sound pressure level of 20 different calling males which were all assumed to be *G. africana* was also measured according to the methodology for each recording, but at a distance of 200 mm beyond the near field from the burrow opening and longitudinal to the long axis on a night between

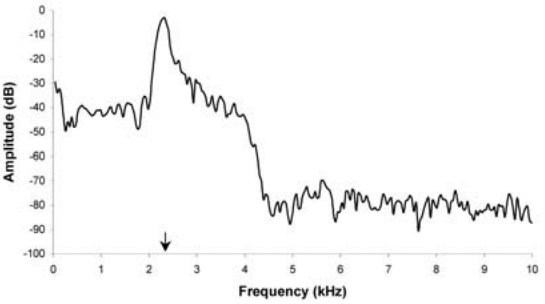

Fig. 3. The power spectrum of a field recorded *G. africana* call (up to 10 kHz), indicating a carrier frequency of approximately 2.3 kHz.

20h00 and 20h30, local time (GMT + 2 hours) in late November 2002. A kikuyu grass area of approximately 300 m^2 including and surrounding of the first tee at Pretoria Country Club was used for measurements. The area sampled had an irrigation program similar to the previous recording sites. Sound level measurements were made with a precision integrating sound level meter (Rion Type NL-14), calibrated by a Rion Type NC-73 sound level calibrator. The equipment was within annual calibration. The sound level meter was used in L_{Aeq} mode, which records the time-weighted average of a series of fast root mean square (RMS) recordings (time constant 125 ms). This gave the A-weighted sound pressure level (dB A scale) (at re. 20 µPa) that was the equivalent continuous level as the fluctuating signal being recorded. A period of approximately 20 s was sufficient to provide a stable level for *G. africana*.

Results

The relationship of call characteristics measured in autumn (March/April) and spring (October/November) of 2002 with soil temperature at 100 mm in the soil profile is represented in Table 1. Soil temperature ranged from 20.7°C to 24.8°C (23.2 ± 1.24°C, mean ± SD) in March/April 2002 recordings and 22.3°C to 26.8°C (23.5 ± 1.16°C, mean ± SD) in October/November 2002 recordings. The data of all the sound characters except syllable period fitted a normal distribution (Kolmogorov-Smirnov test, $P > 0.05$, "Statistica" Version: 5, Statsoft, Inc., 1995) without transformation (Sokal & Rohlf 1997). The syllable period data for both sampling periods was not significantly different from a normal distribution only

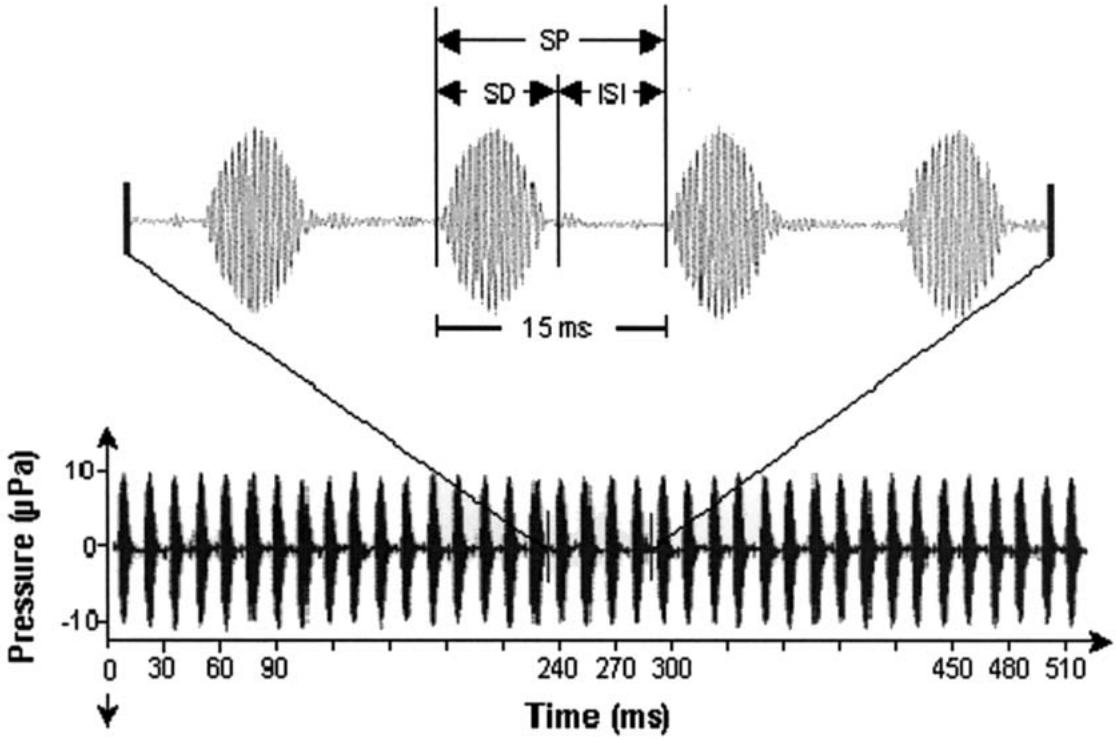

Fig. 4. Oscillogram of a field recorded *G. africana* trilling call over 510 ms. The thickened red lines indicate an approximate eight times shorter temporal scale with the different measurements made. SD = Syllable duration, ISI = Inter syllable interval and SP = Syllable period.

after logarithmic transformation (Sokal & Rohlf 1997) (Kolmogorov-Smirnov test, $P > 0.05$, "Statistica" Version: 5, Statsoft, Inc., 1995).

The multiple regression parametric test ("Statistica" Version: 5, Statsoft, Inc., 1995) showed a highly significant relationship of syllable period, inter syllable interval, syllable repetition rate and duty cycle with soil temperature for both recording periods (Table 1). Carrier frequency variation of *G. africana* males was not significantly related to the tested temperature range. The results show a negative relationship between syllable period and soil temperature for both sampling periods after data were transformed to linear scale before presentation, with soil temperature constantly explaining more than 80% of the variation in the former. The rate of decline in the syllable period was slightly higher in the spring recordings. The syllable duration had no significant relationship with soil temperature. Inter syllable interval was negatively correlated with soil temperature, with R^2 values under 0.50. The rate of decline, however, was slightly higher for the spring recordings relative to those in autumn. The syllable repetition rate was positively related to soil temperature during spring and autumn. In the latter season recordings, the rate of syllable increase was lower than during the spring recordings over a similar range of soil temperatures. Soil temperature was a relatively good predictor (R^2 approximately 0.80) of syllable repetition rate in both recording periods. The duty cycle increased significantly with soil temperature, but with relatively low R^2 values, during both recording periods, respectively. The rate of increase with soil temperature was higher in spring relative to autumn values. Slopes of regression lines should be compared with caution, as they are dependant on the measurement scale.

The values for the different measured and calculated sound characteristics at variable soil temperatures and differences between autumn 2002 and spring 2002 recordings with soil temperature constant are summarized in Table 2. Only syllable repetition rate needed to be arcsine-transformed (Sokal & Rohlf 1997) for all the dependant variables to be normally distributed (Kolmogorov-Smirnov test, $P > 0.05$, "Statistica" Version: 5, Statsoft, Inc., 1995). A multi analysis of variance (MANCOVA, parametric test, Sokal & Rohlf 1997, "Statistica" Version: 5, Statsoft, Inc., 1995), with soil temperature entered as a covariate, was used to determine significant song character differences between the two temporally segregated field recordings.

The results showed that the carrier frequency of *G. africana* males was constant between autumn and spring at approximately 2340 cycles per second (Table 2). The power spectrum (Fig. 3, representative for most songs) graphically represents the carrier frequency and shows a low frequency component and no clear harmonics for *G. africana* males. The spectrogram (Fig. 5) of a general sound recording shows the sound structure during and between syllables. Fig. 5 shows the low frequency observed in the power spectrum was also present between syllables and therefore when no mole cricket sound was produced (Fig. 5).

Syllable duration did not vary significantly between seasons and was usually just longer than nine milli-seconds (Table 2). The syllable period,

TABLE 1. RELATIONSHIP BETWEEN MALE *G. AFRICANA* SONG CHARACTERS AND SOIL TEMPERATURES OF 23.2 ± 1.24°C (MEAN ± SD) AND 23.5 ± 1.16°C (MEAN ± SD) AT A VERTICAL DEPTH OF 100 MM PROFILE IN THE SOIL FOR MARCH/APRIL 2002 (RECORDING 1) AND OCTOBER/NOVEMBER 2002 (RECORDING 2), RESPECTIVELY, AT PRETORIA COUNTRY CLUB.

Data		Regression variable				
Song character	Recording	Slope	Intercept	R^2	*F*	*P*
Carrier frequency (kHz)	1	0.001	2.310	0.0004	0.019	0.891
	2	-0.009	2.569	0.0228	0.931	0.340
Syllable period (ms)	1[2]	-1.067	63.826	0.8092	195.174	0.0000001
	2[2]	-1.127	41.089	0.8139	174.960	0.0000001
Syllable duration (ms)	1	-0.079	11.104	0.0118	0.552	0.461
	2	-0.198	13.702	0.0412	1.7205	0.197
Inter syllable interval (ms)	1[2]	-0.874	25.395	0.4521	37.968	0.0000001
	2[2]	-0.929	27.387	0.3913	25.712	0.000009
Syllable repetition rate (Syllable ms^{-1})	1[2]	0.004	-0.031	0.7926	175.781	0.0000001
	2[2]	0.007	-0.084	0.8406	210.990	0.0000001
Duty cycle (%)	1[2]	3.485	-15.920	0.2755	17.495	0.000128
	2[2]	4.245	-37.130	0.2581	13.915	0.000593

[1]$P < 0.05$.
[2]$P < 0.001$.

TABLE 2. VALUES OF MALE *G. AFRICANA* SONG CHARACTERISTICS RECORDED AT PRETORIA COUNTRY CLUB AT SOIL TEMPERATURES OF 23.2 ± 1.24°C (MEAN ± SD) AND 23.5 ± 1.16°C (MEAN ± SD) AT A VERTICAL DEPTH OF 100 MM IN THE SOIL FOR MARCH/APRIL 2002 (RECORDING 1) AND OCTOBER/NOVEMBER 2002 (RECORDING 2), RESPECTIVELY. SIGNIFICANT DIFFERENCES BETWEEN RECORDINGS WITH SOIL TEMPERATURES CONSTANT ARE SHOWN.

Data		Value		MANCOVA variable	
Song character (Unit)	Recording	Range	Mean ± SD	*F*	*P*
Carrier frequency (kHz)	1	2.198-2.476	2.34 ± 0.067	0.096	0.757
	2	2.161-2.477	2.34 ± 0.075		
Syllable period (ms)[2]	1	12.031-17.061	14.3 ± 1.09	21.226	0.00001
	2	10.455-17.221	14.6 ± 1.45		
Syllable duration (ms)	1	7.340-10.959	9.3 ± 0.91	1.826	0.180
	2	7.372-12.078	9.1 ± 1.13		
Inter syllable interval (ms)[1]	1	2.979-9.607	5.1 ± 1.62	11.548	0.00104
	2	1.912-7.779	5.6 ± 1.72		
Syllable repetition rate (Syllable ms^{-1})[2]	1	0.059-0.083	0.070 ± 0.0061	14.724	0.00024
	2	0.058-0.096	0.069 ± 0.0082		
Duty cycle (%)[1]	1	43.31-78.15	64.9 ± 8.25	7.276	0.00845
	2	48.66-81.72	62.44 ± 0.097		

[1] $P < 0.05$.
[2] $P < 0.001$.

inter syllable interval, syllable repetition rate and duty cycle were significantly different, with soil temperature constant, between the autumn and spring recordings. The syllable period and inter syllable interval were significantly longer and the syllable repetition rate and duty cycle significantly shorter in spring than in autumn, respectively.

During the spring recordings, one individual was recorded at a soil temperature of 21.9°C with the following sound characters (mean ± SD): carrier frequency: 2.638 ± 0.0068 kHz, syllable period: 17.89 ± 0.085 ms, syllable duration: 7.9 ± 0.30 ms. Inter syllable interval, syllable repetition rate, and duty cycle was calculated as (mean ± SD) 10.00 ± 0.217 ms, 0.0559 ± 0.00026 syllables/ms and 44.1 ± 1.47%, respectively.

The sound pressure levels (re. 20 µPa) of *G. africana* varied from 77.6 to 89.8 dB at 200 mm from the burrow. The ambient - and soil temperature (average of five measurements) at the onset of the experiment were 21.5 ± 0.30°C and 23.24 ± 0.112°C, respectively. At the end of the experiment, ambient - and soil temperatures (average of five measurements) were 21.15 ± 0.263°C and 23.03 ± 0.217°C.

DISCUSSION

Gryllotalpa africana males constructed acoustical burrows with one or two horn-shaped openings observed. Two openings may initially have been constructed, but one opening may have collapsed over time. Male African mole crickets started calling just after sunset and, especially during the warm summer months, called until midnight, attracting flying conspecifics and even walking nymphs. Calling activity was generally limited to soil temperatures exceeding 14°C during late August to late May, when conspecifics flew. Initial calling was characterized by a distinctive warm up period. The sound matured from the initial slow erratic trill to a constant trilling call. Some male callers exploited microclimatical conditions near brick walls and concrete slabs. These spatial orientations, which artificially increased soil temperatures, were especially utilized during times of relatively low soil temperatures. Males called singularly, but were usually observed in calling groups as individuals separated by a few meters during stridulation.

Males randomly selected from the field in spring and autumn and acclimatized for one week at L:D 12 h:12 h, which was a relative shorter daily light cycle, and 28 ± 1°C, did not call in the laboratory, suggesting photoperiod as a factor contributing to stridulation activity. This observation may have been biased by the fact that mole crickets were kept in containers, which have been found to influence their behavior (Walker 1979 & Hudson 1988).

Songs of *G. africana* males were produced at sound pressure levels of 77.6 to 89.8 dB and characterized by a carrier frequency of approximately 2.34 kHz with some variation between males. The latter did not vary significantly between autumn and spring and with soil temperature. If the song had a low frequency component, it could not be distin-

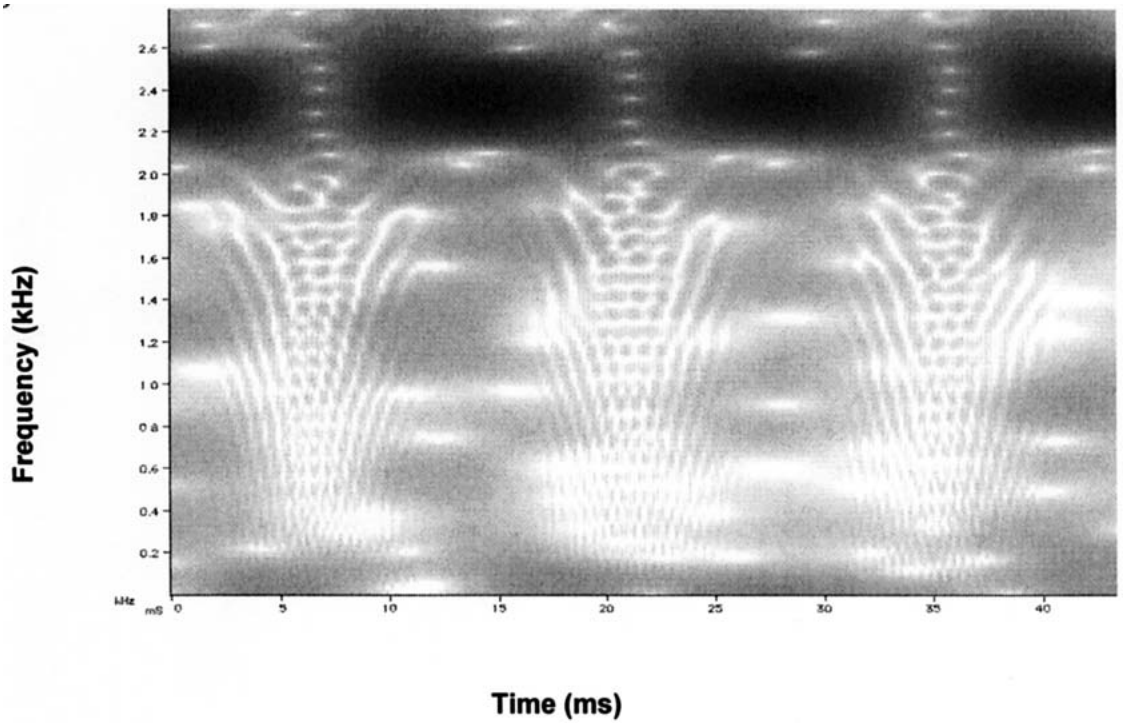

Fig. 5. The spectrogram presenting two complete syllables of a field recorded *G. africana* call (up to approximately 2.7 kHz).

guished from background noise in the current study. Harmonics, which were generally not clearly visible, are usually at a relatively low level in the family (Bennet-Clark 1987). African mole cricket males usually stopped calling, usually less than one minute, when the burrow opening was approached within a one meter radius (personal observation), and the mole cricket was deemed to show some seismic sensitivity. Males in full song were usually less sensitive. Trilling species are generally not very sensitive to substrate vibrations (Bennet-Clarke 1970a; Forrest 1991), although Bennet-Clark (1970a) reported *G. gryllotalpa* to be highly sensitive. Sensitivity may be related to sound pressure level, which may saturate mechano-receptors at high intensities (Bennet-Clark 1970a).

The syllable duration of male *G. africana* calls did not vary significantly between autumn and spring nor with soil temperature, but did show some variation between males. Syllable period was negatively related with soil temperature and varied significantly with soil temperature constant between autumn and spring. Additional sound characters calculated from the syllable period or syllable period and syllable duration, reflected their relationships with the tendencies of the measured variables.

Townsend (1983) reported a mean syllable repetition rate of 49.1-57.8 per second and a mean carrier frequency of 2.1-2.4 kHz for the calling song of *G. africana* based on four recordings. No temperature values or other variables were annotated during these recordings. The calling song frequency of *G. africana* reported from Hawaii is 3.3 kHz, with a syllable repetition rate of 56 per second (Nickle & Castner 1984). Although syllable repetition rates were similar between the two reports, it is not comparable without any temperature information. The carrier frequency values of the present study correspond with that reported by Townsend (1983). Differences in calling song carrier frequency have been used to distinguish between *Gryllotalpa* species (Bennet-Clark 1970b; Nevo & Blondheim 1972). These stridulatory character differences support reports that the Hawaiian species is in fact not *G. africana*. Frank et al. (1998) also stated that the immigrant mole cricket to Hawaii was misidentified as *Gryllotalpa africana*. According to Frank et al. (1998), the species occurring in Hawaii is *G. orientalis*, a species originating from Asia, not Africa.

It appears that a mole cricket species, other than *G. africana*, also inhabited Pretoria Country Club in spring 2002. The distinction of the species was in its higher carrier frequency values. *Gryllotalpa robusta* has a carrier frequency of 1.6 kHz, based on one recording, and *G. parva* has a carrier frequency of 2.9-3.3 kHz, based on two recordings

(Townsend 1983). Hence, the carrier frequency of the unidentified species does not correspond to known values of species occurring in South Africa.

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REFERENCES

BENNET-CLARK, H. C. 1970a. The mechanism and efficiency of sound production in mole crickets. J. Exp. Biol. 52: 619-652.

BENNET-CLARK, H. C. 1970b. A new French mole cricket, differing in song and morphology from *Gryllotalpa gryllotalpa* L. (Orthoptera: Gryllotalpidae). Proc. R. Entomol. Soc. Lond. (B) 39: 125-132.

BENNET-CLARK, H. C. 1987. The tuned singing burrow of mole crickets. J. Exp. Biol. 128: 383-410.

BENNET-CLARK, H. C. 1989. Songs and physics of sound production, pp. 227-261 *In* F. Huber, T. E. Moore, and W. Loher [eds.], Cricket Behavior and Neurobiology, Cornell University Press, Ithaca, NY.

BRANDENBURG, R. L., Y. XIA, AND A. S. SCHOEMAN. 2002. Tunnel architectures of three species of mole crickets (Orthoptera: Gryllotalpidae). Florida Entomol. 85: 383-385.

BROZA, M., S. BLONDHEIM, AND E. NEVO. 1998. New species of mole crickets of the *Gryllotalpa gryllotalpa* group (Orthoptera: Gryllotalpidae) from Israel, based on morphology, song recordings, chromosomes and cuticular hydrocarbons, with comments of the distribution of the group in Europe and the Mediterranean region. Syst. Entomol. 23: 125-135.

CICERAN, M., A-M. MURRAY, AND G. ROWELL. 1994. Natural variation in the temporal patterning of calling song structure in the field cricket *Gryllus pennsylvanicus*: effects of temperature, age, mass, time of day, and nearest neighbour. Can. J. Zool. 72: 38-42.

CORNELL LABORATORY OF ORNITHOLOGY. 1998. Canary. Version 1.2.4. Ithaca.

DE VILLIERS, W. M. 1985. Orthoptera: Ensifera, pp. 86 *In* C. H. Scholtz and E. Holm [eds.], Insects of Southern Africa, Butterworths, Durban.

DOHERTY, J. A., AND J. D. CALLOS. 1991. Acoustic communication in the trilling field cricket, *Gryllus rubens* (Orthoptera: Gryllidae). J. Insect Behav. 4: 67-82.

FORREST, T. G. 1980. Phonotaxis in mole crickets: It's reproductive significance. Florida Entomol. 63: 45-53.

FORREST, T. G. 1983. Calling songs and mate choice in mole crickets, pp. 185-204 *In* D. T. Gwynne and G. K. Morris [eds.], Orthopteran Mating Systems: Sexual Selection in a Diverse Group of Insects, Westview Press, Boulder, CO.

FORREST, T. G. 1991. Power output and efficiency of sound production by crickets. Behav. Ecol. 2:327-338.

FRANK, J. H., T. R. FASULO, AND D. E. SHORT. 1998. Mcricket Knowledgebase. CD-ROM. Institute of Food and Agricultural Sciences. University of Florida, Gainesville.

HILL, P. S. M. 1998. Environmental and social influences on calling effort in the Prairie mole cricket (*Gryllotalpa major*). Behav. Ecol. 9: 101-108.

HILL, P. S. M. 2000. Elements of the acoustic repertoire of the Prairie mole cricket (Orthoptera: Gryllotalpidae: *Gryllotalpa major* Saussure). J. Kansas. Entomol. Soc. 73: 95-102.

HUDSON, W. G. 1988. Field sampling of mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus*): a comparison of techniques. Florida Entomol. 71: 214-216.

KAVANAGH, M. W. 1987. The efficiency of sound production in two cricket species, *Gryllotalpa australis* and *Teleogryllus commodus* (Orthoptera: Grylloidea). J. Exp. Biol. 130: 107-119.

KAVANAGH, M. W., AND D. YOUNG. 1989. Bilateral symmetry of sound production in the mole cricket, *Gryllotalpa australis*. J. Comp. Physiol., A 166: 43-49.

KIM, K. W. 1993. Phonotaxis of the African mole cricket, *Gryllotalpa africana* Palisot de Beauvois. Korean J. Entomol. 32: 76-82 (Abstract cited).

NEVO, E., AND A. BLONDHEIM. 1972. Acoustic isolation in the speciation of mole crickets. Ann. Entomol. Soc. Am. 65: 980-981.

NICKLE, D. A., AND J. L. CASTNER. 1984. Introduced species of mole crickets in the United States, Puerto Rico, and the Virgin Islands (Orthoptera: Gryllotalpidae). Ann. Entomol. Soc. Am. 77: 450-465.

OTTE, D., AND R. D. ALEXANDER. 1983. The Australian Crickets (Orthoptera: Gryllidae). pp. 17: 448-463. The Academy of Natural Sciences of Philadelphia, PA.

SOKAL, R. R., AND F. J. ROHLF. 1997. Biometry. pp. 57, 61-123, 135, 179-260, 392-440, 451-678. W.H. Freeman and Company, New York.

SOUROUKIS, K., W. H. CADE, AND G. ROWALL. 1992. Factors that possibly influence variation in the calling song of field crickets: temperature, time, and male size, age, and wing morphology. Canadian. J. Zool. 70: 950-955.

STATSOFT, INCORPORATED. 1995. Statistica. Version 5.0.

TOWNSEND, B. C. 1983. A revision of the Afrotropical mole-crickets (Orthoptera: Gryllotalpidae). Bull. Br. Nat. Hist. (Ent.) 46: 175-203.

ULAGARAJ, S. M. 1976. Sound production in mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus*). Ann. Entomol. Soc. Am. 69: 299-306.

WALKER, S. L. 1979. Population estimation, damage evaluation and behavioral studies on mole crickets *Scapteriscus vicinus* and *S. acletus* (Orthoptera: Gryllotalpidae). M.S. Thesis, University of Florida, Gainesville.

WALKER, S. L. 1962. Factors responsible for intraspecific variation in the calling song of crickets. Evolution 16: 407-428.

WALKER, T. J., AND D. E. FIGG. 1990. Song and acoustic burrow of the prairie mole cricket, *Gryllotalpa major* (Orthoptera: Gryllidae). J. Kansas Entomol. Soc. 63: 237-242.

WALKER, T. J., AND T. G. FORREST. 1989. Mole cricket phonotaxis: effects of intensity of synthetic calling song (Orthoptera: Gryllotalpidae: *Scapteriscus acletus*). Florida Entomol. 72: 655-659.

DEVELOPMENT OF THE MOST EFFECTIVE TRAP TO MONITOR THE PRESENCE OF THE CACTUS MOTH *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Various trap specifications were evaluated to identify the most effective trap for capturing wild male *Cactoblastis cactorum* (Berg). All traps were baited with virgin female *C. cactorum* and, except for the first comparison of trap type, a standard wing trap was used in all experiments. Although wing traps captured more males than did the other trap types (delta or bucket), the differences were not significant. However, significantly higher numbers of males were captured in wing traps placed 2 m above ground than traps at 1 m or 0.5 m, and wing traps baited with four virgin females caught significantly more males than wing traps baited with a single female. Differences in number of males captured by young and old females were not significant, but more than twice as many males were captured in traps baited with one-day-old females than traps baited with four day old females. In addition, there were no significant differences in number of males caught in unpainted, white, wing traps and wing traps painted one of eight different colors (flat white, black, dark green, fluorescent green, yellow, fluorescent yellow, orange, or blue), although, more males were captured in the unpainted wing traps. The results presented here suggest that the best trap currently available to monitor *C. cactorum* is a standard (unpainted) wing trap, placed at a height of 2.0 m aboveground, and baited with four newly emerged females.

Key Words: population monitoring, trapping, trap design, invasive species, opuntia

RESUMEN

Se evaluaron varios tipos de trampas con el objetivo de identificar la tampa mas efectiva para la captura de machos silvestres de *Cactoblastis cactorum* (Berg). Todas las trampas fueron evaluadas utilizando hembras virgenes de *C. cactorum* como cebo atractivo. Todos los ensayos a excepción del primero (donde se evaluaron tipos distintos de trampas) emplearon la trampa "wing". A pesar de que las trampas wing capturaron un mayor número de machos que ninguna de las otras trampas evaluadas (delta o cubeta) las diferencias en captura de machos no fueron estadisticamente significativas. Tambien, se capturaron un número mayor de machos en las trampas wing colocadas a una altura de 2 metros que en trampas colocadas a una altura de 1 metro o 0.5 metros, y las trampas con 4 hembras como cebo atractivo capturaron un mayor número de machos que las trampas con solamente una hembra. Las diferencias en el número de machos capturados en trampas con hembras de 1 dia de edad en comparación con trampas con hembras de 4 dias de edad no fueron estadisiticamente significativas, pero se capturaron mas del doble del número de machos en trampas con hembras jovenes. No se encontraron diferencias estadiaticas en el número de machos capturados en trampas wing de color estandard o en trampas wing de ocho colores diferentes (blanco mate, negro, verde oscuro, verde fluorescente, amarillo, amarillo fluorescente, naranja o azul), aunque se capturaron mas machos en las trampas wing de color estandard (blanco). Nuestros resultados sugieren que la mejor trampa que se tiene al momento para uso en el monitoreo de *C. cactorum* es la trampa wing de color estandard colocada a una altura de 2.0 metros y con 4 hembras jovenes como cebo atractivo.

Translation provided by the authors.

The cactus moth, *Cactoblastis cactorum* (Berg), was accidentally introduced into Florida in 1989 (Habeck & Bennett 1990; Dickel 1991), and its rapid spread along the Atlantic and Gulf Coasts has heightened concerns about its imminent impact on native *Opuntia* cacti in the southern

United States and Mexico (Johnson & Stiling 1998; Zimmermann et al. 2001). Recent publications suggest that *C. cactorum* is dispersing over a distance of about 50-75 km per year in North America (Stiling 2002; based on data reported in Johnson & Stiling 1998; and Hight et al. 2002). Recent data suggest that the dispersal rate for *C. cactorum* is closer to 160 km per year (S. D. Hight, unpublished data). Establishment of the cactus moth in the southwestern United States and Mexico would likely have serious detrimental effects on the landscape, biodiversity, and stability of native desert ecosystems, and on the vegetable, fruit and forage *Opuntia* industries in these areas (Soberón et al. 2001; Zimmermann et al. 2001, 2004).

The ability to quickly detect new pest infestations, accurately delineate their size and boundaries, and assess the pest's seasonal population trends is of critical importance in the successful application of any pest control strategy. The objective of this research is to develop an optimum monitoring system for adult *C. cactorum* by evaluating various trapping parameters. An efficient adult trapping system is necessary for the development and application of the Sterile Insect Technique (SIT), a control strategy that may be used to study and manage the spread of *C. cactorum* (Carpenter et al. 2001a, b) in North America. SIT is a species-specific pest control tactic that could be used to establish a barrier to prevent further geographic expansion of *C. cactorum* into the western states and Mexico, eradicate new or localized infestations when and where they occur, and/or protect environmentally sensitive areas from attack by the cactus moth. Unfortunately, although female *C. cactorum* produce a pheromone that attracts males (Hight et al. 2003), no synthetic pheromone is currently available to monitor populations of *C. cactorum*.

Hight et al. (2002) were the first to report on the use of sticky traps baited with virgin females to corroborate field damage and better understand the current distribution of *C. cactorum* in Florida and Georgia. More recently, Bloem et al. (2003) showed that sticky traps could be baited with reproductively sterilized females (treated with 200 Gy of gamma radiation) to monitor populations beyond the leading edge without the concern of accidentally establishing a breeding population if females escaped into the environment. The objective of our study was to conduct further field evaluations to ensure that the best monitoring trap is being used to detect the presence of *C. cactorum* and accurately assess its geographical expansion. In this paper we report the results of a series of field experiments conducted during 2003-2004 to evaluate trap types, trap placement heights, and trap colors, as well as the age and number of females for their ability to capture wild male cactus moths. Our results are discussed in the context of developing an area-wide control strategy for *C. cactorum* in North America.

Materials and Methods

Test Insects

Cactoblastis cactorum used in these experiments came from a laboratory colony kept at the USDA-ARS Crop Protection and Management Research Unit in Tifton, GA. Insects were reared on cladodes of *Opuntia stricta* (Haworth) Haworth inside rectangular plastic boxes and maintained at 26°C ± 1°C, a 14:10 (L:D) photoperiod and 70% relative humidity as described in Carpenter et al. (2001b). Cocoons with pupae were collected every two to three days, and pupae were extracted from the cocoons and sorted by gender. Female pupae were placed in a screened cage (30.5 by 30.5 by 30.5 cm) and allowed to emerge at the above mentioned conditions. Emerged adult females were placed inside modified translucent, plastic, film canisters (35 mm, with two 2 by 2-cm screen windows, perforated tops and Velcro fasteners to attach the canister to the top of an insect trap) with a small (1 by 1 cm) piece of *O. stricta*. A moistened cotton dental wick was placed through the perforated canister top to provide moisture to the caged females. Canisters with females were transported to the field in a small cooler and used to bait all treatments in these experiments. All experiments were conducted to coincide with peak flight activity of adult moths (Zimmermann et al. 2004).

Evaluation of Trap Type

Three commercially available insect traps—Pherocon 1-C Wing trap, Pherocon-VI Delta trap (both Trécé Incorporated, Salinas, CA), and Universal moth trap (Unitrap, Great Lakes IPM, Vestaburg, MI)—were evaluated for their effectiveness at capturing wild *C. cactorum* males. Experiments were conducted during the summer of 2003 at coastal locations in Florida and Georgia.

In Florida, three replicates (three traps, one of each type = one replicate) were placed at each of two different coastal locations: Alligator Point (N 29°54', W 84°23') and St. Marks National Wildlife Refuge (NWR) (N 30°04', W 84°10'). Abundant naturally occurring patches of native *O. stricta* that were heavily damaged by *C. cactorum* were present at both sites. Infested *O. ficus-indica* (L.) Miller was also common at Alligator Point as a planted and naturalized species. Hollow metal stakes were placed in the ground in groups of three in the vicinity of infested plants. Within each replicate, metal stakes were separated by no less than 3.0 m from one another and buried so that the top of each stake was at a height of approximately 1.0 m. Each trap was mounted to the top of a stake. Distance between replicates at each location was no less than 50 m. A single virgin female (<48 h post emergence) was used to

bait each trap type. The experiment was initiated on 10 July 2003 and ended on 31 July 2003. Traps were checked every 72 h at which time the number of male *C. cactorum* caught per trap was recorded, traps were re-baited with new virgin females, and the position of each trap was changed in a clockwise manner.

In Georgia, six additional replicates were placed in the proximity of a salt marsh estuary at the southern banks of the Brunswick River in Glynn County, Georgia, west of US Highway 17 (N 31°05', W 81°31'). Within the estuary, a large area of naturally occurring patches of *O. stricta* plants was chosen. One replicate was assigned to each of six patches with cactus plants between 0.5-1.5 m in height. Traps were arranged and serviced as previously described. The experiment was conducted from 2-26 May 2003.

Evaluation of Trap Height

Based on the results from the evaluation of trap type, Pherocon 1-C Wing traps were used in all subsequent experiments. Traps placed at three different heights above ground (2.0, 1.0, and 0.5 m) and were evaluated for their effectiveness at capturing wild *C. cactorum* males. As above, experiments were conducted during the summer of 2003 at coastal locations in Florida and Georgia. In Florida, six replicates (three traps, one trap at each height = one replicate) were placed at St. Marks NWR. Hollow metal stakes were placed in the ground in groups of three close to infested *O. stricta* cactus plants and buried so that the top of each stake was at a height of approximately 1.0 m. Plastic (PVC) poles of a slightly smaller diameter than the hollow metal stakes were cut to the appropriate height and slipped inside each metal stake. A Pherocon 1-C trap was mounted to the top of each plastic pole. Within each replicate, traps were separated by no less than 3.0 m from one another, and distance between replicates at each location was 25-75 m. A single virgin female (<48 h post emergence) was used to bait each trap. The experiment was initiated on 15 July 2003 and ended on 2 August 2003. Traps were checked every 72 h at which time the number of male *C. cactorum* caught at each height was recorded, the positions within replicates of PVC poles with traps were rotated in a clockwise manner, and the traps were re-baited with new virgin females. Six additional replicates were placed at the salt marsh estuary location in Glynn Co., Georgia described above. Six patches with cactus plants between 0.5-1.5 m in height were selected and three hollow metal stakes were placed in the ground within the patches separated by no less than 3.0 m from one another at a height of approximately 1.0 m. As above, one trap was placed on a pole and one pole of each height was placed per patch and checked, serviced, and rotated every 72 h. The experiment was initiated on 29 July 2003 and ended on 28 August 2003.

Evaluation of One Versus Four Females

Pherocon 1-C Wing traps were baited with either one or four virgin female *C. cactorum* (<48 h post emergence) and evaluated for their effectiveness at capturing wild *C. cactorum* males. The experiment was conducted at St. Marks NWR between 24 July 2003 and 5 August 2003. Seven replicates (two traps, baited either with one or four females = one replicate) were completed. Hollow metal stakes were placed in groups of two in close proximity to infested *O. stricta*. Poles were separated by no less than 3.0 m from one another and buried so that the top of each stake was at a height of approximately 1.0 m. A Pherocon 1-C Wing trap was placed on the top of each stake. Distance between replicates at each location was no less than 50 m. Traps were checked every 72 h and the number of male *C. cactorum* caught per trap was recorded, traps re-baited with new virgin females, and trap positions rotated.

Evaluation of Females of Different Ages

Pherocon 1-C Wing traps were baited with single virgin *C. cactorum* females that were either 24 h or 120 h post emergence and evaluated for their effectiveness at capturing wild males. Experiments were conducted at St. Marks NWR from 16-23 April 2003 and at Alligator Point from 28 July-1 August 2003. A total of eleven replicates (each replicate consisted of two traps, one baited with a 24-h-old female and one with a 120-h-old female) were completed: six replicates at St. Marks NWR and five at Alligator Point. As above, hollow metal stakes with a trap on top were placed in the ground in groups of two in the vicinity of infested *O. stricta* and *O. ficus-indica* plants. Within each replicate, metal stakes were separated by no less than 3.0 m and buried so that the top of each stake was at a height of approximately 1.0 m. Distance between replicates at each location was no less than 65 m. Traps were checked at 24 h intervals at which time the number of male *C. cactorum* caught per trap was recorded, traps re-baited with new females, and trap positions rotated.

Evaluation of Trap Color

The outside top and bottom of Pherocon 1-C Wing traps were either left unpainted (controls) or painted with two coats of the following commercially available paints: Gloss White (#7792), Gloss Black (#7779), Dark Hunter Green (#7733), Fluorescent Green Marking (#207464), Sunburst Yellow (#7747), Fluorescent Yellow (#1942) (Rust-Oleum Corp., Vernon Hills, IL), Pumpkin Orange Gloss (#2411), and True Blue Gloss (#1910) (Kry-

lon Products Group, Cleveland, OH). Color selections were based on reported evaluations of trap colors influencing the attraction of various Lepidoptera (Hendricks et al. 1972; Mitchell et al. 1989; Pair et al. 1989; Hendrix & Showers 1990; Lopez 1998; Meagher 2001). Trap surfaces were given two coats of Plastic Primer (Rust-Oleum Corp., Vernon Hills, IL) before painting with experimental colors to increase coverage and adherence of the paint. Experiments were conducted from 14-26 April 2004 on a dike located at St. Marks NWR. Hollow metal stakes were placed in the ground along the dike, separated by no less than 4 m from one another, at a height of approximately 1.5 m, and in the proximity of infested *O. stricta*. Two traps of each color plus two unpainted traps (18 traps total) were deployed randomly in two separate groups (= replicates) on 14 April 2004. All trap bodies were oriented with trap openings in the direction of the prevailing wind. Traps were first baited with two virgin females (<48 h post emergence) on 15 April 2004. Thereafter, traps were serviced, cotton wicks rewetted, and trap location re-randomized every 24 h until 26 April 2004. Females were changed every 48 h. At each trap servicing we noted the status of the female (alive or dead) and the number of male *C. cactorum* captured per trap.

Spectral Reflectance

The spectral reflectance of the painted traps, the standard unpainted trap, and of healthy *O. strica* pads (<1 year-old) was measured with a FieldSpec® Handheld spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO—spectral range of 325-1075 nm). Two readings were taken, one before the traps were placed in the field (15 April 2004 at 1400 h) and one after the experiment was completed (25 May 2004 at 1415 h). Cactus pad reflectance also was measured twice at the same times as the traps.

Statistical Analysis

All statistical analyses were performed with PROC ANOVA (SAS Institute 1989). The effect of trap type (Wing trap, Delta trap, and Universal moth trap) on the number of *C. cactorum* males captured was examined using analysis of variance with trapping date, field site (Brunswick River, Alligator Point and St. Marks), replication, and trap type as sources of variation. Because a significant three-way interaction was found between date, field site, and trap type, the number of males captured was sorted by field site and analyzed with trap type as the main effect, replication as a blocking effect, date as a superblock, and interaction between the trap type and trapping date as an error term. The effect of trap height on the number of male cactus moths captured in Pherocon 1-C Wing traps was analyzed with trap height (2.0, 1.0, and 0.5 m) as the main effect, replication as a blocking effect, field site as a superblock, and interaction between the trap height and field site as an error term. Because no interaction was detected, the data were pooled and ANOVA was conducted by orthogonal contrasts to compare the response of males to the different trap heights. The number of *C. cactorum* males captured in traps baited with one or four virgin females was analyzed with the number of females as the main effect, replication as a blocking effect, date as a superblock, and interaction between the number of females and replication as an error term. The number of *C. cactorum* males captured in traps baited with females that were either 24 or 120 h post emergence was analyzed with trapping date, field site (St. Marks and Alligator Point), and female age as sources of variation. Finally, the effect of trap color on the number of *C. cactorum* males captured in traps was analyzed with trap color as the main effect, replication as a blocking effect, date as a superblock, and interaction between the trap color and date as an error term. All data meet the assumptions for the ANOVA model and were not transformed. Estimates of central tendencies are reported as mean ± standard deviation (SD).

Results

All three trap types (Wing, Delta, and Universal) were successful in capturing males of *C. cactorum* (Table 1). Although the highest mean number of males was captured in the Pherocon 1-C

Table 1. Influence of trap type on the number of male *Cactoblastis cactorum* captured at three different sites. Each trap was baited with a single virgin *C. cactorum* female.

Trap type	Mean (± SD) number of males captured at each trap site**			
	Alligator Point, FL	St. Marks NWR, FL	Brunswick River, GA	Pooled means
Universal moth trap	0.83 ± 1.4	1.67 ± 3.2	0.53 ± 1.2	0.89 ± 1.9
Delta trap	0.39 ± 0.9	1.33 ± 1.7	0.92 ± 1.7	0.92 ± 1.6
Wing trap	1.11 ± 1.6	1.06 ± 2.5	1.33 ± 2.4	1.12 ± 2.2

**Differences among means are not significant, $P > 0.05$ (PROC ANOVA, SAS Institute 1989).

Wing traps tested at Alligator Point, FL and Brunswick River, GA, this type of trap recorded the lowest mean number of captures at St. Marks NWR, FL. Overall, Pherocon 1-C Wing traps captured more males than did the other trap types. However, differences among the means for each trap type were not significant overall or at any of the three field sites. The height at which Pherocon 1-C wing traps were placed above ground (F = 2.73; df = 2, 272; $P < 0.0352$) influenced the number of male *C. cactorum* captured per trap (Table 2). The number of males captured at a height of 2 m was almost twice as many as those captured at 0.5 m. When Pherocon 1-C Wing traps were baited with one or four virgin females, the number of cactus moth males captured was higher (F = 8.18; df = 1, 22; $P < 0.0091$) in traps baited with four females (1.88 ± 2.4) than when baited with single females (0.62 ± 1.1). Furthermore, the age of the female (24 or 120 h post emergence) influenced the number of males captured in Pherocon 1-C Wing traps. Traps baited with a young female (0.84 ± 1.63) captured more than twice the number of males than traps baited with an older female (0.36 ± 0.90). Although the difference between means was not significant, analysis of the data suggests that female age influenced the number of males captured (F = 3.22; df = 1, 72; $P < 0.0768$).

Finally, Pherocon 1-C Wing traps left unpainted (controls) or painted white, black, dark green, fluorescent green, yellow, fluorescent yellow, orange, or blue, and baited with two females were all successful at capturing *C. cactorum* males. Overall, most males were captured in the unpainted Pherocon 1-C Wing traps (1.42 ± 1.8) and the fewest were captured in traps painted gloss white (0.50 ± 0.7), though the differences were not significant (F = 0.71; df = 8, 214; $P <$ 0.6819). In addition, we found no difference in the spectral reflectance readings made on unpainted or painted traps at the beginning compared with the end of the field test. Peak wavelengths for traps of different colors ranged between 0 and 638 nm, and cladode readings were 552 and 759 nm.

TABLE 2. INFLUENCE OF TRAP HEIGHT ON THE NUMBER OF *CACTOBLASTIS CACTORUM* MALES CAPTURED IN PHEROCON 1-C WING TRAPS BAITED WITH A SINGLE VIRGIN FEMALE.

Trap height (m)	Mean (± SD) number of males captured**
0.5	1.13 ± 2.3a
1.0	1.92 ± 3.3b
2.0	2.14 ± 3.3c

**Means followed by a different letter are significantly different, $P \leq 0.05$ (PROC ANOVA using orthogonal contrasts, SAS Institute 1989).

Nevertheless, traps varied widely in their individual spectral reflectance (Table 3). Traps that were painted black, dark green and blue had the lowest reflectance values (all below 50%), as did the cladodes of *O. stricta*. Traps painted orange, fluorescent green, yellow, white and fluorescent yellow, as well as the unpainted control, had high reflectance values (above 50%). Even though the white and the unpainted traps had the same peak wavelength (425 nm) the unpainted trap had the highest spectral reflectance.

DISCUSSION

Trap optimization is of vital importance in developing useful and reliable monitoring systems for pest insects. Habeck and Bennett (1990) were the first to report the presence of *C. cactorum* in the Florida Keys. That initial finding of *C. cactorum* consisted of one adult female collected in a mercury vapor lamp and larvae from infested *O. stricta*. From 1989-2002 cactus moth populations were recorded at different locations along both coasts and at inland sites as far north as Folly Island, SC and as far west as St. Vincent Island, FL (Dickel 1991; Johnson & Stiling 1998; Hight et al. 2002). All of these reported infestations were based on finding damaged cactus plants and/or the presence of immature stages (eggsticks, larvae, pupae) of *C. cactorum* at these locations.

Our group deployed the first virgin female-baited sticky traps used to detect *C. cactorum* in May 2002. As a result of this work, we determined that this insect completes three non-overlapping generations per year in Florida. Each generation has distinct periods of flight activity followed by periods of larval development during which no adults are flying (Zimmermann et al. 2004). In 2003, Hight et al. (2003) reported that the new western limit of *C. cactorum* was at Pensacola Beach, FL, based on visual inspection of highly infested prickly pear plants found during a larval development period. Positive confirmation of *C. cactorum* infestation is relatively easy when larval stages are present and causing heavy damage to *Opuntia* plants. However, determination of the presence of *C. cactorum* can be easily overlooked when infestations are new or small, or the immature stages are not active. Traps baited with adult sexual attractants are effective when the target species is at a low population level (Hanula et al. 1984), the adults are expanding into new areas (Walters et al. 2000), and at times when immature stages are not present (Lalone 1980). Therefore, we wanted to conduct evaluations of our female virgin-baited sticky traps to ensure that the best monitoring tool is being used to detect the presence of *C. cactorum*. While three of the characteristics tested did not reveal significant differences (trap color, trap type, and female age), the trend for each characteristic indicated

TABLE 3. MEAN PEAK WAVELENGTHS (IN NM) AND SPECTRAL REFLECTANCE (IN %) FOR FRESH CLADODES OF *OPUNTIA STRICTA* AND FOR PHEROCON 1-C TRAPS EITHER UNPAINTED (STANDARD) OR PAINTED WITH TWO COATS OF EIGHT DIFFERENT COLORS.

Trap color	Mean peak wavelength (nm)	Reflectance
Gloss Black	0	0.025
Dark Hunter Green	537	0.095
True Blue Gloss	462	0.382
O. stricta cladode	552-759	0.386
Pumpkin Orange Gloss	638	0.667
Fluorescent Green Marking	515	0.926
Sunburst Yellow	557	0.985
Gloss White	425	1.088
Fluorescent Yellow	501	1.219
Unpainted	425	1.286

that unpainted, wing traps baited with 24 h post emergence females resulted in the highest mean capture rate. Considering the overall trap design, the results presented here suggest that the best trap currently available to monitor *C. cactorum* is a standard (unpainted) Pherocon 1-C Wing trap, placed at a height of 2.0 m aboveground, and baited with four newly emerged (24 h post emergence) females.

Trapping studies on other Pyralidae report results similar to ours. For example, Ahmad (1987) and Hanula et al. (1984) found that Pherocon 1-C Wing traps captured the highest number of male almond moth *Cadra cautella* (Walker) and male coneworm *Dioryctria* spp., respectively, when field tested against other trap designs. It is interesting to note that traps placed at a height of 2.0 m captured significantly higher numbers of male *C. cactorum* than did traps placed at 0.5 or 1.0 m. This trap height (2.0 m) is about 0.5-1.0 m higher than the tallest *O. stricta* host plant present at Brunswick River, GA and St. Marks NWR, FL. For some economically important Pyralidae the most effective trap heights are those at or just above host canopy level. This is true for male coneworm captured in pheromone-baited traps (Hanula et al. 1984) and male pickleworm *Diaphania nitidalis* (Stoll) captured in virgin female-baited traps (Valles et al. 1991), but it is not true for almond moth males (Ahmad 1987). One possible explanation for our results is that traps placed at 2.0 m might be "escaping" the competition from pheromone emitting virgin females present in the vegetation. The pheromone plume emitted above the layer of vegetation may be more distinct and attractive to male *C. cactorum*.

Our results suggest that female *C. cactorum* are ready to mate within 24 h of emergence. In recent field experiments Hight et al. (2003) used virgin female *C. cactorum* that were <24 h post emergence in mating tables and observed females emitting pheromone and forming mating pairs. In our Wing traps, the concentration of pheromone from four females is likely to be higher and more attractive to males than the pheromone concentration from a single female *C. cactorum*. Furthermore, the advantage of using more than one female per trap might be that each female actively emits pheromone at slightly different times, thus extending the attractiveness of traps baited with multiple rather than with single females.

Trap color had no influence on male cactus moth captures. However, more males were captured in the unpainted trap which had the highest spectral reflectance of all traps tested. Trap color has been shown to improve trapping efficiency in other economically important Lepidoptera. Knight and Miliczky (2003) found that painted Delta traps captured significantly more male codling moths (*Cydia pomonella* L., Lepidoptera: Tortricidae) than did unpainted traps. In addition, Meagher (2001) showed that traps that had contrasting colors captured more fall armyworm *Spodoptera frugiperda* (J.E. Smith) males than did traps of only one color.

The results presented here give us confidence that we are currently using the most effective trap to detect the presence of cactus moth infestations in North America. Using the best available trapping tool is crucial to determining the best location to deploy a barrier of sterile insects to prevent further westward spread of *C. cactorum*. In addition, and as suggested by Bloem et al. (2003), this same monitoring tool can be deployed beyond the leading edge of infestation by baiting the traps with reproductively sterilized females. While progress is being made on the identification and synthesis of the cactus moth sexual pheromone by colleagues at the USDA-ARS laboratories in Miami and Gainesville FL, no commercial lure is currently available. When experimental pheromone blends do become available, the data presented here will be extremely useful in the field-testing of synthetic lures for cactus moth.

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REFERENCES CITED

AHMAD, T. R. 1987. Effects of pheromone trap design and placement on capture of almond moth *Cadra cautella* (Lepidoptera: Pyralidae). J. Econ. Entomol. 80: 897-900.

BLOEM, S., J. E. CARPENTER, AND K. A. BLOEM. 2003. Performance of sterile *Cactoblastis cactorum* (Lepidoptera: Pyralidae) females in luring males to traps. Florida Entomol. 86: 395-399.

CARPENTER, J. E., K. A. BLOEM, AND S. BLOEM. 2001a. Applications of F_1 sterility for research and management of *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Florida Entomol. 84: 531-536.

CARPENTER, J. E., S. BLOEM, AND K. A. BLOEM. 2001b. Inherited sterility in *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Florida Entomol. 84: 537-542.

DICKEL, T. S. 1991. *Cactoblastis cactorum* in Florida (Lepidoptera: Pyralidae: Phycitinae). Tropical Lepidoptera 2: 117-118.

HABECK, D. H., AND F. D. BENNETT. 1990. *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), a Phycitine new to Florida. Entomology Circular 333. Florida Department of Agriculture and Consumer Services. Division of Plant Industry.

HANULA, J. E., G. L. DEBARR, W. M. HARRIS, AND C. W. BERISFORD. 1984. Factors affecting catches of male coneworms, *Dioryctria* spp. (Lepidoptera: Pyralidae), in pheromone traps in southern pine seed orchards. J. Econ. Entomol. 77: 1449-1453.

HENDRICKS, D. E., J. P. HOLLINGSWORTH, AND A. W. HARTSTACK, JR. 1972. Catch of tobacco budworm moths influenced by color of sex-lure traps. Environ. Entomol. 1: 48-51.

HENDRIX, W. H., III, AND W. B. SHOWERS. Evaluation of differently colored bucket traps for black cutworm and armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 83: 596-598.

HIGHT, S. D., S. BLOEM, K. A. BLOEM, AND J. E. CARPENTER. 2003. *Cactoblastis cactorum* (Lepidoptera: Pyralidae): observations of courtship and mating behaviors at two locations on the gulf coast of Florida. Florida Entomol. 86: 400-407.

HIGHT, S. D., J. E. CARPENTER, K. A. BLOEM, S. BLOEM, R. W. PEMBERTON, AND P. STILING. 2002. Expanding geographical range of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America. Florida Entomol. 85: 527-529.

JOHNSON, D. M., AND P. D. STILING. 1998. Distribution and dispersal of *Cactoblastis cactorum* (Lepidoptera: Pyralidae), an exotic *Opuntia*-feeding moth, in Florida. Florida Entomol. 81: 12-22.

KNIGHT, A. L., AND E. MILICZKY. 2003. Influence of trap colour on the capture of codling moth (Lepidoptera; Tortricidae), honeybees, and non-target flies. J. Entomol. Soc. B.C. 100: 65-70.

LALONE, R. S. 1980. Pest management of leafrollers in caneberries grown in Oregon. Acta Hortic. 112: 135-141.

LOPEZ, J. D., JR. 1998. Evaluation of some commercially available trap designs and sex pheromone lures for *Spodoptera exigua* (Lepidoptera: Noctuidae). J. Econ. Entomol. 91: 517-521.

MEAGHER, R. L. 2001. Collection of fall armyworm (Lepidoptera: Noctuidae) adults and nontaget Hymenoptera in different colored unitraps. Florida Entomol. 84: 77-82.

MITCHELL, E. R., H. R. AGEE, AND R. R. HEATH. 1989. Influence of pheromone trap color and design on capture of male velvetbean caterpillar and fall armyworm moths (Lepidoptera: Noctuidae). J. Chem. Ecol. 15: 1775-1784.

PAIR, S. D., J. R. RAULSTON, A. N. SPARKS, S. R. SIMS, R. K. SPRENKEL, G. K. DOUCE, AND J. E. CARPENTER. 1989. Pheromone traps for monitoring fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), populations. J. Entomol. Sci. 24: 34-39.

SAS INSTITUTE. 1989. SAS user's guide. SAS Institute, Cary, NC.

SOBERÓN, J., J. GOLUBOV, AND J. SARUKHAN. 2001. The importance of *Opuntia* in Mexico and routes of invasion and impact of *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Florida Entomol. 84: 486-492.

STILING, P. 2002. Potential non-target effects of a biological control agent, prickly pear moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), in North America, and possible management actions. Biological Invasions 4: 273-281.

VALLES, S. M., J. L. CAPINERA, AND P. E. A. TEAL. 1991. Evaluation of pheromone trap design, height, and efficiency for capture of male *Diaphania nitidalis* (Lepidoptera: Pyralidae) in a field cage. Environ. Entomol. 20: 1274-1279.

WALTERS, M. L., R. T. STATEN, AND R. C. ROBERSON. 2000. Pink bollworm integrated management using sterile insects under field trial conditions, Imperial Valley, California, pp. 201-206 *In* Keng-Hong Tan [ed.], Area-Wide Control of Fruit Flies and Other Insect Pests: Joint Proceedings of the International Conference in Area-Wide Control of Insect Pests and the 5th International Symposium of Fruit Flies of Economic Importance, 1998, Penang, Malaysia, Penerbit Universiti Sains Malaysia, Pulau Pinang, 782 pp.

ZIMMERMANN, H. G., S. BLOEM, AND H. KLEIN. 2004. Biology, history, threat, surveillance and control of the cactus moth, *Cactoblastis cactorum*. IAEA, Vienna, Austria. 40pp.

ZIMMERMANN, H. G., V. C. MORAN, AND J. H. HOFFMANN. 2001. The renowned cactus moth, *Cactoblastis cactorum*: its natural history and threat to native *Opuntia* in Mexico and the United States of America. Diversity and Distributions 6: 259-269.

INFLUENCE OF DIET AND METHYL EUGENOL ON THE MATING SUCCESS OF MALES OF THE ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The chief objective of this study was to determine whether the inclusion of protein in the adult diet influences male mating success in the oriental fruit fly, *Bactrocera dorsalis* (Hendel). Previous studies on this species have shown that ingestion of methyl eugenol (ME) greatly enhances male mating performance. Accordingly, we also examined the interaction between adult diet and ME and investigated whether this chemical boosts the male mating success independent of diet. In trials conducted in field tents, we compared the mating frequency of control, protein-fed males (no ME) versus males (1) deprived of protein during the entire adult life, the pre-maturation period, or the post-maturation period and (2) not provided ME or provided ME 1 day before testing. Males deprived of protein completely or when immature (1-12 days old) obtained very few matings (<5% total matings) with or without ME feeding. Males provided protein as immature adults but deprived of protein as mature adults (>12 days old; no ME) also were competitively inferior to control males but achieved a significantly higher proportion (37%) of total matings than males in the preceding treatments. ME exposure boosted the mating success of these males slightly (40% of total matings) such that their mating frequency was not significantly different from control males. Additional tests showed that for treated males fed protein their entire adult life short-term (30 h) food deprivation resulted in a significant decrease in mating success, and feeding on ME did not boost the mating frequency of the food-deprived males. The implications of our findings for controlling *B. dorsalis* via sterile male releases are discussed.

Key Words: Diptera, Tephritidae, oriental fruit fly, diet, methyl eugenol, mating behavior

RESUMEN

El objetivo principal de este estudio fue el determinar si la inclusión de proteina en la dieta del adulto tiene una influencia en el éxito de apareamiento de los macho en la mosca oriental de fruta *Bactrocera dorsalis* (Hendel). Estudios anteriores sobre esta especie han mostrado que la ingestión de eugenol metil (EM) mejora sustancialmente la capacidad del apareamiento del macho. De consiguiente, nosotros tambien examinamos la interación entre la dieta del adulto y EM e investigamos si esta química aumentará el exito de apareamiento del macho independiente de la dieta. En pruebas llevadas a cabo en tiendas de campaña en el campo, nosotros comparamos la frecuencia de apareamiento del grupo control, machos alimentados con próteina (no EM) versus machos 1) privados de próteina durante su vida de adulto entera, el periodo de pre-maduración, o el periodo del pos-maduración y 2) no proveidos con EM o no proveidos con EM un dia antes de la prueba. Los machos completamente privados de proteina o que eran inmaduros (1-12 dias de edad) conseguieron muy pocos apareamientos (<5% de los apareamientos total) con o sin alimentar de EM. Los machos proveidos con proteina en el estadio inmaduro pero privados de proteina como adultos maduros (>12 dias de edad, sin EM) fueron inferiores en competir con los macho del grupo control pero lograron obtener una proporción significativamente mas alta (37%) de apareamientos total que los machos en los tratamientos anteriores. La exposición a EM aumentó el éxito del apareamiento de estos machos ligeramente (40% de todos los apareamientos) de tal manera que la frecuencia de apareamiento no fue significativamente diferente de la frecuencia de los machos en el grupo de control. Pruebas adicionales mostraron que, para los machos tratados con alimentos con próteina para toda su vida del adulto, la privación de alimentos a un corto plazo (30 h) resultaron en una diminución significativa en el éxito de apareamiento, y la alimentación de EM no aumentó la frecuencia de apareamiento de los machos privados de alimentos. Se discuten las implicaciones de nuestros hallazgos para controlar *B. dorsalis* por la via de liberación de machos estériles.

The quality of the adult diet, particularly the inclusion of protein, may influence the ability of male insects to attract females and obtain copulations as shown for certain species of Orthoptera (Andrade and Mason 2000; Scheuber et al. 2003) and Diptera (Stoffolano et al. 1995; Droney 1998). Recent data from two economically important genera of tephritid fruit flies (Diptera: Tephritidae) likewise reveal an important effect of dietary protein on male signaling and mating success (for

protein effects on female tephritids, see Cangussu & Zucoloto 1995; Jacome et al. 1999). Working with a wild population of Mediterranean fruit flies (medflies), *Ceratitis capitata* (Wiedemann), in Israel, Yuval et al. (1998) found that sexually active males (i.e., those participating in leks) contained more sugar and protein than sexually inactive (resting) males. In a follow-up study, Kaspi et al. (2000) observed wild medflies on field-caged host trees and found that protein-fed males spent more time emitting pheromone (see Papadopoulos et al. 1998 for a similar finding) and achieved more matings than protein-deprived males. In a study of wild medflies in Hawaii, Shelly et al. (2002) observed no effect of dietary protein on the frequency of pheromone calling but found that protein-fed males attracted more females than protein-deprived males and had a significant advantage in mating competition over protein-deprived males (see also Shelly & Kennelly 2002). In addition to this focus on dietary protein, several studies (Papadopoulos et al. 1998; Shelly & Kennelly 2003) have demonstrated the adverse effect of short-term food deprivation (18-24 h) on signaling activity and mating success of wild medfly males.

The effect of diet on the mating behavior of wild males also has been examined in several *Anastrepha* species. Aluja et al. (2001) reared wild males of 4 *Anastrepha* species on sugar, a mixture of sugar and protein, open fruit, or a mixture of sugar and bird feces and then compared males in the different diet treatments with respect to signaling activity and mating frequency. In 3 of the species, males fed the combination of sugar and protein displayed the highest frequency of pheromone-calling, and in two of these, males fed only sugar called significantly less frequently than conspecifics fed other diets. In the same 3 species, males fed the sugar and protein diet obtained significantly more matings than males fed other diets. In only 1 species, *A. ludens* (Loew), did diet have no effect on male sexual activity.

The chief objective of the present study was to determine whether dietary protein has a similarly strong effect on male mating success in the Oriental fruit fly, *Bactrocera dorsalis* (Hendel). Previous studies on *B. dorsalis* (e.g., Shelly & Dewire 1994) have demonstrated that ingestion of methyl eugenol (ME), a powerful male attractant occurring naturally in many plant species (Tan & Nishida 1996), dramatically increases male mating success, apparently owing to the incorporation of ME metabolites in the sex pheromone (Nishida et al. 1987) and the subsequently heightened attractiveness of the olfactory signal (Shelly & Dewire 1994). The effect of ME feeding on male reproductive performance has been investigated only within the context of protein-rich, adult diets. Consequently, we also examined the interaction between diet and ME feeding to determine whether ME feeding similarly boosts the mating performance of protein-deprived males. Finally, in light of the aforementioned results for the medfly, we measured the effect of food deprivation on the mating success of *B. dorsalis* males. Implications of our findings for controlling *B. dorsalis* via sterile male release are discussed. For background information on the mating behavior of *B. dorsalis*, see Fletcher (1987) and Shelly and Kaneshiro (1991).

Materials and Methods

Study Flies

All flies used in the present study were from a laboratory colony started with 600-800 adults reared from papayas (*Carica papaya* L.) collected near Hilo, HI. The colony was maintained in a screen cage (l:w:d, $1.2 \times 0.6 \times 0.6$ m) and provided a mixture (3:1, wt:wt) of sugar (sucrose) and hydrolyzed protein (enzymatic yeast hydrolysate) and water *ad libitum* and papayas for oviposition. Infested papayas were held over vermiculite, and the pupae were sifted from vermiculite 16-18 days later. Adults used in mating trials were separated by sex within 48 h of eclosion, well before reaching sexual maturity at ≈ 15 days of age (TES, unpublished data), and held in screen-covered, plastic buckets (volume 5 liters with a cloth sleeve to allow transfer of flies, food, and water; 100-125 individuals per bucket) with ample food and water. Flies were held at 24-28°C and 60-90% RH and received natural and artificial light under a 12:12 (L:D) photoperiod. When used in the experiments, the flies were 3-7 generations removed from the wild.

Competitive Mating Tests

Four mating experiments were conducted in which males subject to different rearing regimes competed for copulations. All tests used mature individuals of both sexes (males: 21-25 days old; females: 21 - 29 days old). In each experiment, the control males were fed the sugar-protein mixture (hereafter termed the SP diet) and water continuously during their entire adult life (as were all females tested) and never given access to ME. The food mixture was placed in a small Petri dish (5 cm diameter), and water was provided via a cotton wick emerging from a covered, plastic cup. Both the food and water were changed every 5-7 days.

Treated males were subject to the following conditions.

Experiment 1a: Treated males were fed sugar-agar exclusively throughout their entire adult life (i.e., these males were protein-deprived) and were not given access to ME. No water cup was provided. The sugar-agar diet (hereafter termed the

S diet) was prepared following the recipe of the California Preventative Release Program and included water (84.66% by weight), sugar (14.57%), agar (0.76%), and methyl parabin (0.01%). A block of the sugar-agar (l:w:h, approximately 6 × 3 × 2 cm) was placed directly on the screen-cover of the bucket and was replaced every 2-3 days.

Experiment 1b. Same as above, except that treated males were given access to ME (supplied by FarmaTech Intl. Corp., Fresno, CA). In this, and all subsequent experiments involving ME exposure, treated males were given access to ME on the day before testing. Using a micropipette, we applied 100 µl of ME to a cotton wick (held vertically by insertion through a hole in the lid of a plastic cup), which was then placed in a bucket holding 80-100 males. The chemical was introduced between 1000-1300 hours and removed 1 h after placement. Feeding activity was not monitored in this study, but in a previous study (Shelly 1997) over 90% of mature males were found to feed on ME within a 1-h interval.

Experiment 2a. Treated males were fed the S diet from 1-12 days of age and were then provided the SP diet until tested (hereafter termed the S-SP diet). As males do not attain sexual maturity until about 15 days of age (TES, unpublished data), this treatment provided sugar only to immature males but sugar and protein to sexually mature males. Treated males were not given access to ME.

Experiment 2b. Same as experiment 2a, except that treated males were given access to ME following the above protocol.

Experiment 3a. Treated males were fed the SP diet and water from 1-12 days of age and were then provided the S diet until tested (hereafter termed the SP-S diet). Here, we reversed the treatment of experiment 2a and provided sugar and protein to immature males but sugar only to mature males. Treated males were not given access to ME.

Experiment 3b. Same as experiment 3a, except that treated males were given access to ME following the above protocol.

Experiment 4a. Treated males were fed the SP diet during their entire life but were starved for approximately 30 h prior to testing. Treated males were not given access to ME. As noted above, *B. dorsalis* is sexually active at dusk, and consequently we removed the food (but not the water cup) at noon on the day before testing.

Experiment 4b. Same as experiment 4a, except that treated males were fed ME. In this case, ME was introduced at 1100 hours, and starvation was initiated upon termination of the exposure period.

Competitive mating trials were conducted between August 2004-January 2005, in field cages (3 m diameter; 2.5 m height) at the USDA-ARS laboratory, Honolulu (air temperature: 25-30°C; RH: > 60%). The tents each contained two artificial trees whose leaves resembled those of *Ficus benjamina* L. Each tree was approximately 2 m tall and bore 700-800 leaves. Males perform the normal suite of reproductive behaviors on these trees, and matings occur as frequently as on potted host trees (TES, unpublished data). Groups of 75 control males, 75 treated males, and 75 females were released approximately 2 h before sunset (between 1600-1700 hours depending on the test date). For a given trial, we marked both control and treated males 1 day prior to testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. The cages were monitored from 1 h before sunset until approximately 30 min after sunset with a flashlight. Mating pairs were collected in vials and returned to the laboratory where the males were identified. Eight replicates were conducted for experiments 1-3, and 10 replicates were conducted for experiment 4.

Non-Competitive Mating Tests

Because adult diet had a profound effect on male mating success (see below), we conducted a series of non-competitive mating tests to assess the mating propensity of males exposed to different dietary regimes. To expedite data collection, these tests were run in our laboratory by placing 10 females (maintained on the SP diet) and 10 males of a given treatment in plexiglass cages (l:w:h, 40 × 30 × 30 cm) approximately 2 h before sunset and scoring the number of matings 2-3 h after sunset. Cages were placed near a west-facing window and exposed to natural light (room lights were extinguished when flies were placed in the cages). When tested, males were 21-24 days old, and females were 23-29 days old. For a given male treatment, 6-10 cages were run per day over 3-5 different days for a total of 30 replicates (cages) per treatment.

Non-competitive mating tests were conducted with males subject to treatments identical to those described above for the competitive mating trials and included control males and treated males as described for experiments 1-3. Test males were subject to the following conditions.

Experiment 5. Males were fed the SP diet during their entire life and were not given access to ME.

Experiment 6a. Males were fed the S diet during their entire life and were not given ME.

Experiment 6b. Same as above, but males were given access to ME.

Experiment 7a. Males were fed the S-SP diet and were not given access to ME.

Experiment 7b. Same as above, but males were given access to ME.

Experiment 8a. Males were fed the SP-S diet and were not given access to ME.

Experiment 8b. Same as above, but males were given access to ME.

Diet and Male Survival

Survival was compared among males maintained on the SP, S, S-SP, or SP-S diets. For each diet, 20 males (1 day old) were placed in screen cages (30 cm cubes) with the appropriate diet under the laboratory conditions described above. All diets were presented in small Petri dishes; water was provided with all diets except the S diet. Food and water were changed every 2-3 days. Cages were checked midday every day for 40 days, and dead males were removed during the daily observations. Ten cages were observed for each diet type.

Statistical Analyses

For the competitive mating experiments, we first compared the number of matings obtained per replicate between control and treated males using a t-test as the assumptions of normality and equal variances were met in nearly all cases (the exceptions were experiments 1a, 1b, and 2a, respectively, and here data were normalized via $\log_{10}$ [x + 1] transformation). Because there was significant variation in the total number of matings observed per replicate among the different experiments ($F_{7, 60} = 5.2$, $P < 0.001$, ANOVA; presumably owing to slight variation in weather conditions), we compared mating performance among the different treatments using the proportion (arc sine transformed) of the total matings obtained per replicate in an ANOVA. For the noncompetitive mating experiments, mating numbers were compared among male treatments by the Kruskal-Wallis test (a logarithmic transformation failed to normalize the data). For male survivorship data, the number of survivors was plotted against time for the different treatments, and slopes of the regression lines were compared by ANCOVA following Zar (1996). As described below, multi-group comparisons involving mating frequency or survivorship revealed significant variation in all cases, and consequently the Tukey test was run to identify significant differences in specific pair wise comparisons. For survivorship, the test statistic q was calculated according to Zar (1996).

RESULTS

Competitive Mating Tests

Results of the competitive mating trials are presented in Table 1. The most striking finding was the dramatic, negative effect of protein-depri-

TABLE 1. EFFECTS OF ADULT DIET, METHYL EUGENOL (ME), AND STARVATION ON THE MATING SUCCESS OF *B. dorsalis* MALES IN COMPETITIVE MATING TRIALS.

Experiment	Treatment		Starvation	Male type	Number of Matings[1]	t[2]	% Total Matings[3]
	Diet	ME					
1 a	S	no	no	Control	20.4 (3.7)	12.3***	
				Treated	0.8 (1.1)		2.8[a]
1 b	S	yes	no	Control	21.0 (3.4)	16.6***	
				Treated	0.7 (0.7)		3.2[a]
2 a	S-SP	no	no	Control	17.1 (2.4)	13.2***	
				Treated	0.9 (1.0)		4.7[a]
2 b	S-SP	yes	no	Control	13.6 (3.4)	0.2[NS]	
				Treated	13.0 (6.2)		47.0[b]
3 a	SP-S	no	no	Control	18.9 (4.6)	3.3**	
				Treated	11.2 (4.5)		37.5[b]
3 b	SP-S	yes	no	Control	16.8 (4.8)	1.2[NS]	
				Treated	13.2 (5.2)		40.5[b]
4 a	SP	no	yes	Control	12.8 (4.5)	2.8*	
				Treated	7.3 (3.8)		35.6[b]
4 b	SP	yes	yes	Control	17.1 (8.9)	2.5*	
				Treated	8.7 (5.5)		35.9[b]

[1]Values represent average numbers (±1 sd) of matings per replicate and average proportion of total matings obtained by treated males; 8-10 replicates were conducted per experiment.

[2]Tests compare control and treated groups for a given experiment, where significance levels are: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; [ns]not significant.

[3]Proportions followed by the same letter are not significantly different at $P = 0.05$, tukey test.

vation on male mating success. Males fed the S diet their entire life obtained, on average, less than 1 mating per replicate without (experiment 1a) or with (experiment 1b) prior access to ME. Males maintained on the S-SP diet and not provided ME (experiment 2a) had a similarly low mating success. Treated males in these experiments accounted for a similar proportion (3%-5%) of the total matings. In contrast to the S diet, males fed the S-SP diet and then exposed to ME (experiment 2b) displayed a significant increase in mating success and were, in fact, competitively equivalent to control males.

Males maintained on the opposite dietary regime, SP-S, and denied access to ME (experiment 3a) were competitively inferior to control males, but they obtained, on average, a significantly higher proportion of matings per replicate than S-SP males denied ME (experiment 2a; 38% vs. 5%, respectively). Exposure to ME increased the mating success of males reared on the SP-S diet (experiment 3b), and the mean number of matings obtained by these males was similar to that observed for the control males. Relative to the S-SP diet used in experiment 2, however, the effect of ME exposure with the SP-S diet was slight: males fed the SP-S diet and given ME or denied ME accounted for a similar proportion of the total matings (40% versus 37%, respectively).

Starvation had a strong negative effect on mating frequency. Control males had a mating advantage over starved males (previously maintained on the SP diet) independent of ME feeding (experiments 4a and 4b). Starved males denied or provided ME obtained the same proportion (36%) of the total matings.

Non-Competitive Mating Tests

Results from the non-competitive mating tests mirrored those described above for the competitive tests (Table 2). Significant variation in mating frequency was observed among male treatments (H = 64.8, $df = 6$, $P < 0.001$). Males on the S and S-SP diets exhibited mating frequencies that were significantly lower than those observed for the SP or SP-S diets. Over the 60 replicates involving the S diet (i.e., experiments 6a and 6b combined), we observed a total of only 3 matings. Similarly, only 5 matings were recorded for males fed the S-SP diet independent of ME exposure. Males on the SP diet displayed the highest mating frequency, although this was not significantly different from males on the SP-S diet after ME exposure.

Male Survivorship

Significant variation in slope existed among the different diet treatments ($F_{3, 28} = 5.9$, $P = 0.007$), with survival rate being greatest for the SP diet, lowest for the S diet, and intermediate for the SP-S and S-SP diets (Fig. 1). Multiple comparison tests revealed that survival rate for the SP diet was significantly greater than for the S or SP-S diets but not significantly different from the S-SP diet. No significant differences were detected in pair wise comparisons among the S, SP-S, and S-SP diets. The simple linear regression equations were: SP: y = 18.5-0.16×, $r^2 = 0.89$; S: y = 18.6-0.31×, $r^2 = 0.97$; S-SP: y = 18.1-0.22×, $r^2 = 0.90$; SP-S: y = 18.8- 0.26×, $r^2 = 0.94$.

TABLE 2. EFFECTS OF ADULT DIET AND METHYL EUGENOL (ME) ON THE MATING FREQUENCY OF *B. DORSALIS* MALES IN NON-COMPETITIVE MATING TRIALS.

	Treatment		Number of Matings	
Experiment	Diet	ME	Mean	Median[1]
5	SP	no	3.8 (1.5)	4[a]
6 a	S	no	0.1 (0.2)	0[c]
6 b	S	yes	0.1 (0.2)	0[c]
7 a	S-SP	no	0.2 (0.5)	0[c]
7 b	S-SP	yes	0.2 (0.4)	0[c]
8 a	SP-S	no	2.6 (1.3)	3[b]
8b	SP-S	yes	2.9 (1.4)	3[ab]

[1]Mean (±1 sd) and median numbers of matings are given over 30 replicates per experiment. Medians followed by a common letter are not significantly different at $P = 0.05$, Tukey test.

DISCUSSION

The present study demonstrates a strong effect of diet quality on the mating success of *B. dorsalis* males. The inclusion of hydrolyzed protein in the

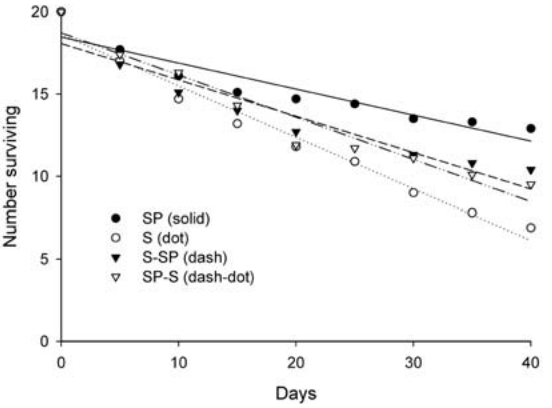

Fig. 1. Survivorship of males maintained on different diet regimes. Points represent means of 10 cages per diet treatment.

adult diet was very important for mating; males fed the sugar-agar gel exclusively achieved very few matings in competitive or even non-competitive conditions. The low number of copulations observed in the non-competitive situation further suggests that males were not sexually active at all or were producing signals unattractive to females. Interestingly, males reared on the S diet until day 12 and then switched to the SP diet for 8-12 days before testing (and not given ME) performed as poorly as males reared on the S diet exclusively, and this was evident in both competitive and noncompetitive situations. Males subject to the opposite treatment (SP-S) had a lower mating success than control males in the competitive trials but nonetheless accounted for a much higher proportion of the total matings than did the S or S-SP males. Results from the starvation treatment further revealed that males deprived of the PS diet for a single day had reduced mating success relative to males continuously fed the same diet.

Dietary protein appears to have a greater effect on mating success for wild males of *B. dorsalis* than for wild males of *C. capitata*. Whereas sugar-fed males of *B. dorsalis* rarely mated, sugar-fed males of *C. capitata* accounted for approximately 33% (Kaspi et al. 2000) and 40% (Shelly et al. 2002) of the total matings in competition with protein-fed males. The impact of dietary protein varied greatly among *Anastrepha* species (Aluja et al. 2001). As in *B. dorsalis*, males of *A. serpentina* (Wiedemann) and *A. striata* Schiner that were fed a sucrose solution only mated very infrequently (<10% total matings). In contrast, based on non-competitive mating trials, males of *A. obliqua* (Macquart) fed sugar only still mated at nearly half the rate as protein-fed males, and no diet effect at all was detected for *A. ludens*.

The present findings provide only a broad description of the impact of adult nutrition on male mating success, and additional tests are required to determine more specifically the effect of intermittent protein-feeding on male performance. In particular, future studies should address the question of whether, among sexually mature males, mating success declines with time since the last protein meal. In other words, while the present study showed that both (i) a sugar-only, post-maturation diet and (ii) starvation from a protein-rich diet reduced male mating success, it did not investigate the impact of more realistic feeding regimes, where, for example, males may locate sugar-rich foods more or less continuously but protein-rich foods only irregularly.

In addition to demonstrating the importance of dietary protein, our study also provided information regarding the interaction between diet and ME on male mating success. Among continuously fed males, ME had no effect on mating performance for males that were fed the S diet exclusively and only a slight effect on males fed the SP-S diet. However, ME dramatically boosted mating success under the S-SP diet: ME-fed males obtained an average of 47% of all matings per replicate (experiment 2b) compared to only 5% for ME-deprived males (experiment 2a). Although ME exposure under the S-SP diet yielded equivalent mating success between treated and control males, prior studies (Shelly & Dewire 1994; Shelly & Nishida 2004) have shown that, among males fed the SP diet exclusively, individuals provided with ME obtain approximately 2/3 of all matings in competition against ME-deprived individuals. Thus, ME exposure under the S-SP diet apparently compensated for a low quality diet, but it did not confer a mating advantage as evident under the SP dietary regime. Interestingly, ME feeding did not offset the adverse effect of starvation (from the SP diet; experiment 4b) on male mating success. It thus appears that the ability to locate protein-rich food is essential for *B. dorsalis* males, because temporary deprivation of the SP diet not only reduced mating performance but also eliminated the potential benefit associated with ME ingestion.

In conclusion, our study is potentially relevant to control programs, such as that ongoing in Thailand, that utilize the sterile insect technique (SIT) to suppress or eradicate infestations of *B. dorsalis*. Our tests were performed exclusively on flies from a relatively "young" laboratory colony, and additional work is required to determine whether the composition of the adult diet similarly influences the mating competitiveness of males from long-established, mass-reared strains of *B. dorsalis*. In the Mediterranean fruit fly, for example, inclusion of protein in the adult diet invariably results in increased mating success of wild males (Yuval et al. 1998; Kaspi et al. 2000; Shelly et al. 2002), whereas the results for mass-reared males have been inconsistent (compare Blay & Yuval 1997; Kaspi & Yuval 2000 with Shelly and Kennelly 2002; Shelly and McInnis 2003). Clearly, however, if adult diet similarly affects mass-reared *B. dorsalis* males, an effective use of the SIT would require inclusion of protein in the pre-release diet.

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References Cited

Aluja, M., I. Jacome, and R. Macias-Ordonez. 2001. Effect of adult nutrition on male sexual performance in four neotropical fruit fly species of the genus *Anastrepha* (Diptera: Tephritidae). J. Insect Behav. 14: 759-775.

Andrade, M. C. B., and A. C. Mason. 2000. Male condition, female choice, and extreme variation in repeated mating in a scaly cricket, *Ornebius aperta* (Orthoptera: Gryllidae: Mogoplistinae). J. Insect Behav. 13: 483-497.

Blay, S., and B. Yuval. 1997. Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). Anim. Behav. 54: 59-66.

Cangussu, J. A., and F. S. Zucoloto. 1995. Self-selection and perception threshold in adult females of *Ceratitis capitata* (Diptera, Tephritidae). J. Insect Physiol. 41: 223-227.

Droney, D. C. 1998. The influence of the nutritional content of the adult male diet on testis mass, body condition and courtship vigour in a Hawaiian *Drosophila*. Funct. Ecol. 12: 920-928.

Fletcher, B. S. 1987. The biology of dacine fruit flies. Annu. Rev. Entomol. 32: 115-144.

Jacome, I., M. Aluja, and P. Liedo. 1999. Impact of adult diet on demographic and population parameters in the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). Bull. Entomol. Res. 89: 165-175.

Kaspi, R., and B. Yuval. 2000. Post-teneral protein feeding improves sexual competitiveness but reduces longevity of mass-reared sterile male Mediterranean fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 93: 949-955.

Kaspi, R., P. W. Taylor, and B. Yuval. 2000. Diet and size influence sexual advertisement and copulatory success of males in Mediterranean fruit fly leks. Ecol. Entomol. 25: 279-284.

Nishida, R., K. H. Tan, M. Serit, N. H. Lajis, A. M. Sukari, S. Takahashi, and H. Fukami. 1987. Accumulation of phenylpropanoids in rectal glands of males of the Oriental fruit fly, *Dacus dorsalis*. Experientia 44: 534-536.

Papadopoulos, N. T., B. I. Katsoyannos, N. A. Kouloussis, A. P. Economopoulos, and J. R. Carey. 1998. Effect of adult age, food, and time of day on sexual calling incidence of wild and mass-reared *Ceratitis capitata* males. Entomol. Exp. Appl. 89: 175-182.

Scheuber, H., A. Jacot, and M. W. G. Brinkhof. 2003. Condition dependence of a multicomponent sexual signal in the field cricket *Gryllus campestris*. Anim. Behav. 65: 721-727.

Shelly, T. E. 1997. Selection for non-responsiveness to methyl eugenol in male oriental fruit flies (Diptera: Tephritidae). Florida Entomol. 80: 248-253.

Shelly, T. E., and A. M. Dewire. 1994. Chemically mediated mating success in male oriental fruit flies, *Bactrocera dorsalis* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 87: 375-382.

Shelly, T. E., and K. Y. Kaneshiro. 1991. Lek behavior of the oriental fruit fly, *Dacus dorsalis*, in Hawaii (Diptera: Tephritidae). J. Insect Behav. 4: 235-241.

Shelly, T. E., and S. S. Kennelly. 2002. Influence of male diet on male mating success and longevity and female remating in the Mediterranean fruit fly (Diptera: Tephitidae) under laboratory conditions. Florida Entomol. 85: 572-579.

Shelly, T. E., and S. S. Kennelly. 2003. Starvation and the mating success of wild male Mediterranean fruit flies (Diptera: Tephritidae). J. Insect Behav. 16: 171-179.

Shelly, T. E., and D. O. McInnis. 2003. Influence of adult diet on the mating success and survival of male Mediterranean fruit flies (Diptera: Tephritidae) from two mass-rearing strains on field-caged host trees. Florida Entomol. 86: 340-344.

Shelly, T. E., and R. Nishida. 2004. Larval and adult feeding on methyl eugenol and the mating success of male oriental fruit flies, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Entomol. Exp. Appl. 112: 155-158.

Shelly, T. E., S. S. Kennelly, and D. O. McInnis. 2002. Effect of adult diet on signaling activity, mate attraction, and mating success in male Mediterranean fruit flies (Diptera: Tephritidae). Florida Entomol. 85: 150-155.

Stoffolano, J. G., E. N. Tobin, J. Wilson, and C. M. Yin. 1995. Diet affects insemination and sexual activity in male *Phormia regina* (Diptera: Calliphoridae). Ann. Entomol. Soc. Am. 88: 240-246.

Tan, K. H., and R. Nishida. 1996. Sex pheromone and mating competition after methyl eugenol consumption in the *Bactrocera dorsalis* complex, pp. 147-153 *In* B. A. McPheron and G. J. Steck [eds.], Fruit Fly Pests: A World Assessment of Their Biology and Management. St. Lucie Press, Delray Beach, FL.

Yuval, B., R. Kaspi, S. Sloush, and M. Warburg. 1998. Nutritional reserves regulate male participation in Mediterranean fruit fly leks. Ecol. Entomol. 23: 101-105.

Zar, J. H. 1996. Biostatistical analysis. 3rd ed. Prentice Hall, Upper Saddle River, NJ.

INFESTATION OF STORED SAW PALMETTO BERRIES BY *CADRA CAUTELLA* (LEPIDOPTERA: PYRALIDAE) AND THE HOST PARADOX IN STORED-PRODUCT INSECTS

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ABSTRACT

The almond moth, *Cadra cautella* (Walker), is a common storage pest known to infest a wide range of dried plant materials, and it has been recorded from a warehouse in Florida during storage of dried passion-flower (*Passiflora incarnata* L.) and dried saw palmetto berries *Serenoa repens* (Bartram) Small. Its status as a pest of stored saw palmetto was confirmed by trapping in a second warehouse used solely for storage of this commodity. The moth occurred in high numbers, captures were closely associated with stacks of bagged berries, and trap catch was very low after the stacks were consolidated under a tarp and fumigated. Yet the results of laboratory rearing on saw palmetto suggested that *C. cautella* has little ability to infest this commodity—development was protracted and highly variable in duration, mortality was high, and pupal weight was low. This sort of contradiction in host suitability, which we refer to as the "host paradox," may be widespread among stored-product insects but has seldom been reported and almost never studied. Published reports suggest that the solution lies partly in dietary supplementation through fungivorous, saprophagous, or carnivorous feeding, although more subtle factors also are suggested. Even cursory observations of the host paradox should be reported to document frequency of occurrence and perhaps stimulate studies directed toward solutions. Such studies would inevitably provide better understanding of population dynamics, which would, in turn, lend support to better management of insects in commercial storage situations.

Key Words: development, saw palmetto, stored-product insects, almond moth

RESUMEN

La polilla de la almendra, *Cadra cautella* (Walker), es una plaga común de almacén que es conocida por infestar un amplio rango de material de plantas secas, y ha sido registrada de una bodega en Florida durante el almacimiento de la flor seca de maracuya (*Passiflora incarnata* L.) y las moras secas del palmito *Serenoa repens* (Bartram) Small. Su estado como una plaga del palmito fue confirmado por medio de trampas puestas en una segunda bodega usadas solamente para el almacimiento de esta material. La polilla ocurre en altos numeros, las capturadas fueron asociadas estrechamente con los apilados de moras embolsadas y el número de polillas capturadas en trampas fue muy bajo después de que los estantes fueron cubiertos bajo una tarpa y fumigados. Aún así los resultos de criar la polilla sobre el palmito en el laboratorio sugiere que *C. cautella* tiene poca abilidad de infestar este material—el desarrollo fue prolongado y altamente variable en duración, la mortalidad fue alta, y el peso de la pupa fue muy bajo. Esta clase de contradicción en el mantenimiento desarrollo sobre el hospedero, lo cual referimos como la "paradoja del hospedero", puede ser ampliamente distribuida entre los insectos de productos almacenados pero raramente ha sida reportada y casi nunca estudiada. Los informes publicados sugieron que la solución consiste parcialmente en la suplementación de dieta por medio de la alimentación fungivora, saprofaga, o carnivora, aunque se sugiere que hay unos factores mas sutiles. Las observaciones precipitadas de la "paradoja del hospedero deben ser reportadas para documentar la frecuencia de ocurrencia y tal vez con ello estimular estudios dirigidos hacia el encuentro de soluciones. Tales estudios definitivemente proveeran un mejor entendimento de la dinámica de la población, lo cual en cambio, dara apoyo para el mejor manejo de insectos en productos comerciales almacenados.

The almond moth, *Cadra cautella* (Walker), is a common, often serious pest of dried plant materials. It has been recorded from cereal grains and their products, dried fruit, nuts, oilseeds, pulses, and cacao (Richards & Thomson 1932), and also has been reported from dried passion-flower and dried saw palmetto berries (Arbogast et al. 2002).

Saw palmetto, *Serenoa repens* (Bartram) Small, is one of many plants that provide botanicals for the production of pharmaceuticals and

herbal supplements. It occurs in the southeastern coastal plain of the United States from South Carolina to Mississippi, where it is a common element of pine flatwoods, mesic hammocks, prairies, and scrubs (Bennett & Hicklin 1998). An extract of the berries, or the ground berries themselves, are reported to be useful in maintaining prostate and urinary tract well-being (Koch 2001), and they are sold for this purpose as a dietary supplement. The berries are harvested by hand from their native habitats during late summer and fall. They are dried to a moisture content of 8-14%, bagged in burlap, and stored pending shipment to end processors. During storage, they are subject to infestation by a variety of stored-product insects.

Arbogast et al. (2002) described the species composition and spatial distribution of an insect population infesting a botanicals warehouse at Mascotte, FL, which was used alternately for storage of dried saw palmetto berries and dried passion-flower (maypop), *Passiflora incarnata L.* *Cadra cautella* was the dominant species, comprising 47% of the insect population when saw palmetto was in storage. To confirm the status of *C. cautella* as a pest of stored saw palmetto, we studied the spatial relationship between adult moths and stacks of bagged berries in a second warehouse used solely for storage of this commodity. We also conducted laboratory experiments to test the hypothesis that successful infestation of the dried berries can be explained entirely by the suitability of this host for growth and development of the moth. The present paper reports the results of these studies and examines the results in the context of host range in stored-product insects, especially the utilization of marginal host commodities.

Materials and Methods

Warehouse Studies

The warehouse, located in La Belle, FL, recently had been acquired for storage of saw palmetto berries and previously had been used for processing and storage of fresh peppers (green bell peppers, jalapeno peppers, etc.). The building was a modern steel structure 40 m wide by 55 m long with a covered dock along the east side (Fig. 1). The only walled areas within the building were restrooms and a small office in one corner and a large cold storage room in another. There were sixteen propane-fired batch driers and a belt drier adjacent to the dock, which served as a work area during drying operations and also for storage of crates and bagged saw palmetto debris, which is used as mulch. In addition to saw palmetto, the building itself contained processing equipment and a few pallets, crates, and burlap bags. The dried and bagged berries were stacked, usually six bags high, on wooden pallets, and the pallets were in turn stacked one on top of another. In January 2001, when we first visited the warehouse, most of the stacks were inside the cold storage room, but there were several small stacks elsewhere (Figs. 1 and 2A). The door to the cold storage room was kept closed, even though the refrigeration was no longer used. In late April, all of the stacks were moved and consolidated in preparation for a commercial fumigation with phosphine (Fig. 2 B-C), which was done under a tarp covering the stacked bags. The fumigation began on April 27, and the warehouse was closed until May 2.

We made three, 24-h trapping runs, one in January, one in late April immediately before the fumigation, and one in early May immediately after the fumigation. The traps were pheromone-baited sticky traps (SP-Locator traps with SP Minimoth pheromone dispensers, AgriSense-BCS Ltd., Pontypridd, Mid Glamorgan, UK). Trap locations were specified in rectangular coordinates with the origin at the southwest corner of the warehouse. The number and configuration of trap locations (Fig. 2A-C) varied slightly. Trap density was increased in areas with stacked commodity, but the spacing between traps was always > 4 m, the measured active space of the lures (Mankin et al. 1999). When trap locations were not occupied by stacks, moth traps were placed 1.2 m above the floor, attached by means of Velcro either to the walls of the warehouse or to the tops of wooden stakes supported by stands on the floor. Otherwise, moth traps were placed on top of the stacked bags or attached to walls slightly above the stacks. Placement of traps under these circumstances was necessarily imprecise, but we estimated that all were within <1 m of their designated coordinates. The stacks ranged in height from slightly over 1 m to about 4.5 m, and trap height varied accordingly.

To determine the spatial distribution of trap catch for each trapping run, the x, y-coordinates of the trap positions and the corresponding numbers of moths captured were entered in Surfer 7 (Golden Software, Golden, CO) for contour analysis. This software posted observed trap catch to the appropriate coordinates on a floor plan of the warehouse, which had been entered as a base map, and then created a denser grid of trap catch values by interpolation, using radial basis functions (with the multiquadric function). This method of interpolation produces good representation of most small data sets (<250 observations) (Golden Software 1999).

Laboratory Studies

Laboratory cultures of *C. cautella* were established in January 2001 with adults collected from the La Belle warehouse. The moths were reared on

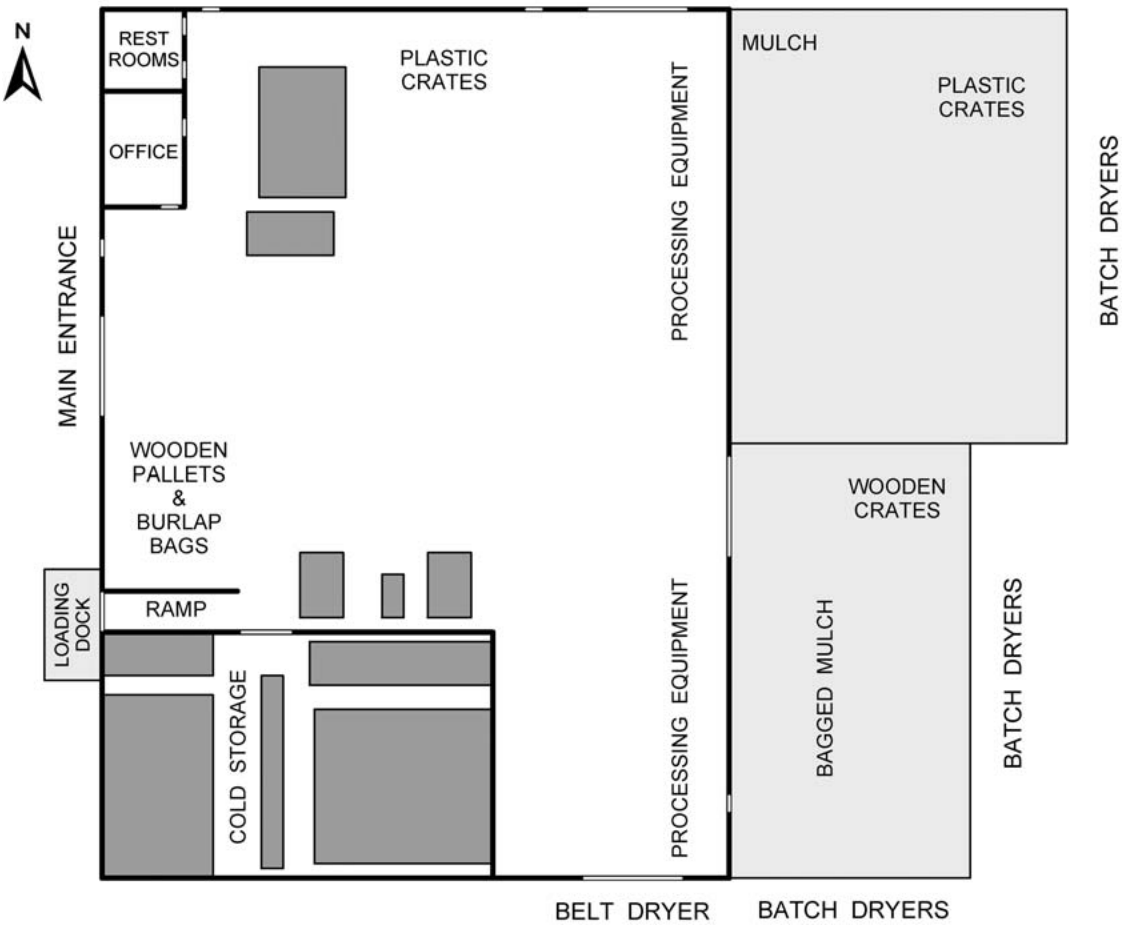

Fig. 1. Floor plan of a botanicals warehouse at La Belle, FL used for drying, bagging, and storing saw palmetto berries. The light gray areas represent docks outside the building. The dark gray areas are stacks of bagged berries on pallets.

a standard laboratory diet (Silhacek and Miller 1972) consisting of ground Gaines dog meal (10%), rolled oats (4%), white cornmeal (26%), whole wheat flour (23%), wheat germ (2%), brewers' yeast (5%), glycerol (16%), and honey (14%). Cultures were maintained in a walk-in environmental chamber (EGC, Chagrin Falls, OH) at 27 ± 1°C and 60 ± 5% RH under a 12:12 h light-dark cycle. All experimental procedures, except moisture determinations, were conducted within the chamber.

To compare developmental time, pupal weight, and survivorship on saw palmetto with that on the laboratory diet, larvae were reared individually on 8 ml of ground saw palmetto in 16 ml Wheaton sample bottles (Wheaton Scientific, Millville, NJ). Ventilation was achieved by a screen-covered opening (2 cm diam) in each bottle cap. The screen consisted of disks cut from extra fine phosphor-bronze cloth (Hillside Wire Cloth Co., Bloomfield, NJ) to fit inside the caps.

Dried saw palmetto berries from the La Belle warehouse were coarsely ground in a blender. These ground berries and the laboratory diet were equilibrated to the relative humidity of the environmental chamber, for which purpose a quantity of each diet sufficient to complete all experiments, as well as provide 6 samples for moisture determination, was placed in clear polystyrene boxes (19.5 × 14.0 × 7.0 cm) (Tri-State Plastics, Dixon, KY) with screened holes (7 cm diam) in the lids. A shallow layer of diet was placed in each box and held in the environmental chamber for one week, during which time it was stirred daily. The diets were then retained in the boxes until they were used for rearing or moisture determination. Moisture content of the diets was determined by an air-oven method, AACC Method 44-15A (American Association of Cereal Chemists 2003), when the first experiment was set up and again when the last experiment was completed.

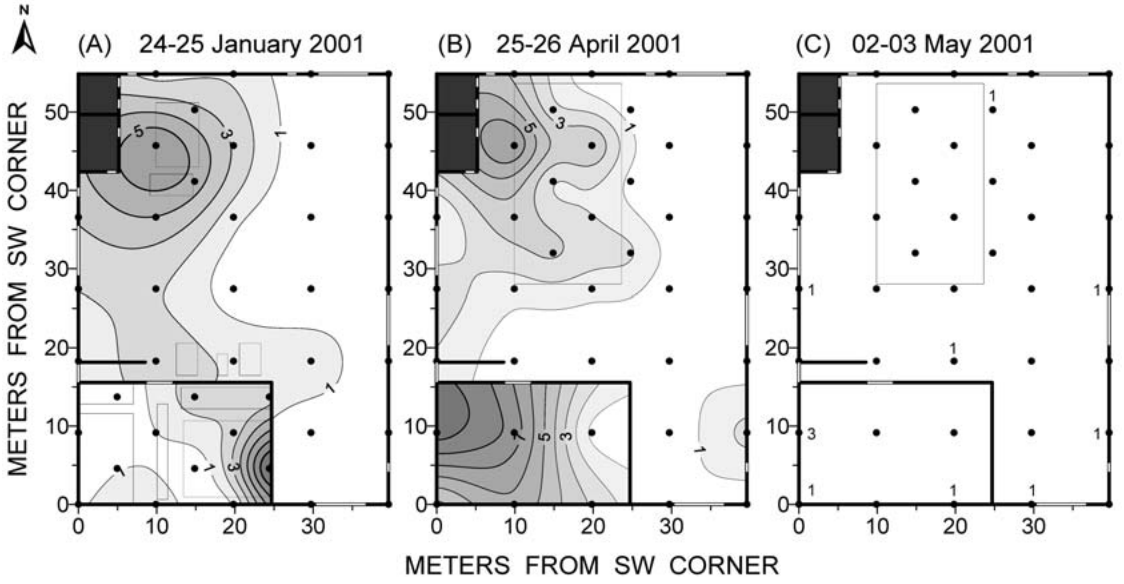

Fig. 2. Spatial distribution of *C. cautella* in a botanicals warehouse as indicated by trap catch of adult males. Trap locations are indicated by dots and locations of stacked bags by rectangular outlines. Contour lines represent total trap catch during a 24-h trapping period. Following fumigation (C), there were too few captures for contour analysis, so trap catch is indicated by numerals adjacent to trap locations. The total number of moths captured during each of the three trapping runs was (A) 65, (B) 78, and (C) 11.

About 8 ml of moisture-equilibrated diet were placed in each of 100 bottles, 50 bottles per diet, and one newly hatched larva was added to each bottle. This procedure was repeated three times at weekly intervals. To obtain larvae, eggs were collected over a 6-8 hr period, confined in petri dishes without food, and held in the environmental chamber. After 2 days, the eggs were checked for hatching every 2-3 hr during the workday, and newly hatched larvae were transferred with a camel hair brush to the bottles with diets. Enough larvae were obtained in this manner during a single day to set up one experiment.

The bottles with larvae were allowed to remain undisturbed for 2 wk and then examined daily for pupation and adult eclosion. When a pupa became fully tanned (light brown), it was gently removed from its cocoon with soft forceps, weighed on an ultra-microbalance (Type UMT2, Mettler Instrument Corp., Hightstown, NJ) with readability to 0.0001 mg, and then returned to the bottle. Observations were continued until all the insects either emerged or died.

Combined data for males and females were used in running statistical tests. Differences in mean survivorship and in pupal weight were analyzed by *t*-tests, the latter after square-root transformation of the data. The data for developmental period failed tests for normality and equal variance, which could not be corrected by any of the common transformations, so the data were analyzed by the Mann-Whitney test (SigmaStat 3.0, Systat Software, Inc., Richmond, CA), which compares median values.

Results

Warehouse Study

The spatial distribution of adult *C. cautella,* as indicated by trap catch, showed a close association of the moths with the stacks of berries (Fig. 2). In late January 2001, most of the berries were stored inside the closed cold room, and the stacks covered much of the floor space (Fig. 2A). The highest trap catch occurred within this room along the east wall. The remaining captures were concentrated on two stacks near the office and rest rooms with captures extending from there to three very small stacks just outside the cold room. Again in late April the highest trap catch occurred inside the cold room (Fig. 2B), from which the berries had been removed a few days earlier. Following fumigation (Fig. 2C), only 11 moths were trapped, 5 inside the cold room and the remainder widely scattered about the warehouse. None were trapped on the stack and only one immediately adjacent to the stack.

Laboratory Study

The moisture content of the laboratory diet (mean ± SE) was 15.2 (± 0.04)% at the beginning of the experiment and 13.9 (± 0.05)% at the end.

The moisture content of the saw palmetto ranged from 10.2 (± 0.07)% initially to 10.1 (± 0.08)% at the end.

Development of *C. cautella* on saw palmetto was protracted and highly variable in duration, mortality was high, and pupal weight was low (Table 1). The median developmental period on saw palmetto was significantly longer (by 60 d) than on laboratory diet (Mann-Whitney test; $P < 0.01$) and pupal weight was significantly lower ($t = 21.60$; $df = 153$; $P < 0.01$). Survival on saw palmetto also was lower than on laboratory diet ($t = 11.75$, $df = 4$, $P < 0.01$). The mean survival rate (±SE) based on three groups of 50 insects was 89.3 ± 4.4 on laboratory diet and 16.7 (±4.4) on saw palmetto. Although no adult moths were weighed or otherwise measured, those that developed on saw palmetto were obviously smaller than those reared on the laboratory diet, as would be expected given the difference in pupal weight.

Discussion

The high numbers of *C. cautella* in the La Belle warehouse, the close association of trap captures with stacks of bagged berries, and the very low trap catch after fumigation of the consolidated stack, provide conclusive evidence that *C. cautella* infests saw palmetto during storage and is, in fact, a major pest. Yet in the laboratory, *C. cautella* displayed little ability to attack saw palmetto. Its protracted development, with high larval mortality and low pupal weight, suggest that populations would increase little, or even decline, on saw palmetto. This contradiction, a phenomenon we term the "host paradox," may be quite widespread among stored-product insects, but has seldom been reported and almost never studied.

Storage insects typically have broad host ranges that include dry commodities of both plant and animal origin, but these commodities differ markedly in their ability to support population growth (Arbogast 1991). Variation in host suitability is well illustrated by studies of the cigarette beetle, *Lasioderma serricorne* (F.), which is an extremely polyphagous stored product insect with reported hosts including tobacco, spices, cereals, pulses, seeds, nuts, dried fruit, dried vegetables, cocoa beans, coffee beans, yeast, bamboo, copra, ginger, licorice root, herbarium specimens, dried fish, fish meal, and meat meal (Howe 1957). In a comparative study of selected plant products, Howe (1957) found a range of 80 d in mean developmental period and 97% in survival rate (at 30°C). In a study of spices, LeCato (1978) observed a range of 100 d in median developmental period and 88% in survivorship (at 28°C). Products such as whole peas, curry powder and chili powder are relatively poor diets for *L. serricorne* and do not support rapid population growth. An additional example of variation in host suitability is provided by a study of three stored-product moths on dried fruits, almonds, and carobs (Cox 1975). The mean developmental period of *C. cautella*, for instance, ranged from 35 d on almonds to 84 d on raisins (30°C). The developmental period of *C. cautella* on the standard laboratory diet in the present study was much shorter than on any of the commodities reported by Cox (1975), even though temperature was lower (Table 1).

Clearly, some commodities are only marginally suitable as hosts for storage insects. They barely support development, but yet these same commodities may become seriously infested in commercial storage. Awareness of this paradox is important in evaluating the potential pest status of stored-product insects; host studies in the laboratory may not reveal true pest potential under commercial storage conditions.

The reasons for this discrepancy and the occurrence of the host paradox may lie partly in dietary supplementation through fungivorous, saprophagous, and carnivorous feeding, which are known to enhance development and population growth, especially on poor host commodities. The foreign grain beetle, *Ahasverus advena* (Waltl), for example, feeds on a wide variety of stored products, including grain and cereal products, but usually oc-

Table 1. Development of *C. cautella* from egg hatch to adult eclosion on laboratory diet and on dried Saw Palmetto Berries at 27 ± 1°C and 60 ± 5% RH.

	Laboratory diet		Saw palmetto	
Development	*n*	Mean ± SE	*n*[1]	Mean ± SE
Males				
Period (d)	55	23.1 ± 0.2	18	84.7 ± 2.9
Pupal wt (mg)	55	14.5 ± 0.25	16	5.3 ± 0.32
Females				
Period (d)	72	23.4 ± 0.2	7	91.6 ± 7.9
Pupal wt (mg)	74	19.9 ± 0.30	7	6.0 ± 0.45

[1]*n,* the number of insects on which the mean is based.

curs in large numbers only when a commodity is moldy. Woodroffe (1962) found that *A. advena* cannot breed successfully on rolled oats or whole wheat flour in the absence of visible mold unless yeast or wheat germ are added, suggesting that some cereal products are deficient in an essential nutrient that can be provided by molds, yeast, or wheat germ. The Indianmeal moth, *Plodia interpunctella* (Hübner), may also require fungal supplementation for development on some of its hosts. This moth is a pest of stored raisins, but fails to develop on raisins in the laboratory unless the grapes used to produce the raisins are inoculated before drying, with a particular fungus known to infect grapes in the field (Charles Burks, personal communication). Burks hypothesized that the conidia of this fungus support neonate larval development, while raisins alone do not. Other storage insects are known to supplement their diet by saprophagous feeding. Thus, population growth of the red flour beetle, *Tribolium castaneum* (Herbst), on several cereal grains increases when dead eggs or adults of *P. interpunctella* are added to the grain (LeCato & Flaherty 1973; LeCato 1975a). Supplementation with dead moth eggs and adults also increases population growth of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), on peanuts, but not on more suitable diets such as corn, wheat, or rice (LeCato 1973). Facultative predation also has been shown to enhance population growth of storage insects. Population growth of *T. castaneum*, for instance, is increased on some commodities by predation on the immature stages of *O. surinamensis* (LeCato 1975b).

Fungus feeding, saprophagy, and predation may sometimes provide the answer to the host paradox, but less obvious factors also can be involved. This is evidenced by the association between field infestation of carobs by the pyralid moth *Ectomyelois ceratoniae* (Zeller) and the ability of *C. cautella* to infest carobs in storage (Dobie 1978). Carob pods crack as they are ripening on the tree and become infested by *E. ceratoniae*, which is attracted by a mold growing in the cracked pods. After harvest, carobs may become infested by a variety of stored-product insects, including *C. cautella*. Dobie (1978) found that previous field infestation has a marked effect on the suitability of carobs as a food for *C. cautella*, an effect that cannot be attributed to mechanical damage, because the carobs used in his experiments were coarsely ground. Ovipositing females and first instars of *C. cautella* show a preference for previously infested carobs, on which survival is much higher. Dobie postulated that factors directly associated with field infestation must render carobs attractive to *C. cautella* and aid in larval development.

Development of storage insects in commercial settings on commodities that fail to support development in the laboratory may occur more commonly than suggested by the meager number of references in the literature. Under-reporting of this phenomenon may arise from a reluctance to publish data perceived to be negative. An incisive discussion on the importance of negative data and the potential consequences for scientific theory of withholding publication can be found in an essay by Gould (1993). Even cursory observations of the host paradox should be reported to document frequency of occurrence and perhaps stimulate studies directed toward solutions. Such studies would inevitably provide better understanding of population dynamics, which would in turn lend support to better management of insects in commercial storage situations.

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References Cited

American Association of Cereal Chemists. 2003. Approved methods of the American Association of Cereal Chemists (AACC). AACC, St. Paul, MN. 1, 200 pp.

Arbogast, R. T. 1991. Beetles: Coleoptera, pp.131-176 *In* J. R. Gorham [ed.], Ecology and Management of Food Industry Pests. FDA Bulletin 4. Association of Official Analytical Chemists, Arlington, VA. 595 pp.

Arbogast, R. T., P. E. Kendra, R. W. Mankin, and R. C. Mcdonald. 2002. Insect infestation of a botanicals warehouse in north-central Florida. J. Stored Prod. Res. 38: 349-363.

Bennett, B. C., and J. R. Hicklin. 1998. Uses of saw palmetto (*Serenoa repens*, Arecaceae) in Florida. Econ. Bot. 52: 381-393.

Cox, P. D. 1975. The suitability of dried fruits, almonds, and carobs for the development of *Ephestia figulilella*, *E. calidella* and *E. cautella*. J. Stored Prod. Res. 11: 229-233.

DOBIE, P. 1978. The effects of previous field infestation upon *Ephestia cautella* (Walker) and *Lasioderma serricorne* (F.) infesting carobs. J. Stored Prod. Res. 14: 35-39.

GOLDEN SOFTWARE. 1999. Surfer 7 user's guide. Golden Software, Inc., Golden, CO. 619 pp.

GOULD, S. J. 1993. Cordelia's Dilemma. Nat. Hist. 2/93: 10-18.

HOWE, R. W. 1957. A laboratory study of the cigarette beetle, *Lasioderma serricorne* (F.) (Col., Anobiidae) with a critical review of the literature on its biology. Bull. Entomol. Res. 48: 9-56 + 2 plates.

KOCH, E. 2001. Extracts from fruits of saw palmetto (*Sabal serrulata*) and roots of stinging nettle (*Urtica dioica*): viable alternatives in the medical treatment of benign prostatic hyperplasia and associated lower urinary tract symptoms. Planta Med. 67: 489-500.

LECATO, G. L. 1973. Sawtoothed grain beetle: Population growth on peanuts stimulated by eating eggs or adults of the Indianmeal moth. Ann. Entomol. Soc. Amer. 66: 1365.

LECATO, G. L. 1975a. Red flour beetle: Population growth on diets of corn, wheat, rice, or shelled peanuts supplemented with eggs and adults of the Indianmeal moth. J. Econ. Entomol. 68: 763-765.

LECATO, G. L. 1975b. Predation by red flour beetle on sawtoothed grain beetle. Environ. Entomol. 4: 504-506.

LECATO, G. L. 1978. Infestation and development by the cigarette beetle in spices. J. Georgia Entomol. Soc. 13: 100-105.

LECATO, G. L., AND B. R. FLAHERTY. 1973. *Tribolium castaneum* progeny production and development on diets supplemented with eggs or adults of *Plodia interpunctella*. J. Stored Prod. Res. 9: 199-203.

MANKIN, R. W., R. T. ARBOGAST, P. E. KENDRA, AND D. K. WEAVER. 1999. Active spaces of pheromone traps for *Plodia interpunctella* (Lepidoptera: Pyralidae) in enclosed environments. Environ. Entomol. 28: 557-565.

RICHARDS, O. W., AND W. S. THOMSON. 1932. A contribution to the study of the genera *Ephestia* Gn. (including *Strymax* Dyar), and *Plodia* Gn. (Lep. Phycitidae), with notes on parasites of the larvae. Trans. R. Entomol. Soc. London. 80: 169-250.

Silhacek, D. L., AND G. L. Miller. 1972. Growth and development of the Indian meal moth. *Plodia interpunctella* (Lepidoptera: Phycitidae), under laboratory mass-rearing conditions. Ann. Entomol. Soc. Amer. 65: 1084-1087.

WOODROFFE, G. E. 1962. The status of the foreign grain beetle, *Ahasverus advena* (Waltl) (Col., Silvanidae), as a pest of stored products. Bull. Entomol. Res. 53: 537-540.

DO WING MARKINGS IN FRUIT FLIES (DIPTERA: TEPHRITIDAE) HAVE SEXUAL SIGNIFICANCE?

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ABSTRACT

The patterned wings of tephritid fruit flies often are moved in complex manners during sexual encounters. However, there are few cases of sexual dimorphism, and wing movements also may occur in non-sexual contexts. There was no evidence that enhancing or obliterating the patterns on the wings of male Caribbean fruit flies, *Anastrepha suspensa* (Loew), had any effect on their sexual success. There is convergence in wing patterns with another Dipteran family, the distantly related Bombyliidae. Additional studies of mating systems with this family might illuminate the significance of similar wing patterns in tephritids.

Key Words: sexual selection, mate choice, sexual signal, insect vision, Bombyliidae, courtship, crypsis

RESUMEN

Las alas moteadas de las moscas tefrítidas de fruta a menudo son movidas de una manera compleja durante los encuentros sexuales. Sin embargo, hay pocos casos de dimorfismo sexual, y los movimientos de las alas pueden occurir en un contexto no sexual. No hubo evidencia que el incremento o eliminación de los patrones sobre las alas de los machos de la mosca de la fruta del Caribe, *Anastrepha suspensa* (Loew), tuvo un efecto sobre el exito sexual. Hay una convergencia de los patrones de alas con otra familia en el orden Diptera, la familia Bombyliidae, que esta relacionada lejanamente. Estudios adicionales de los sistemas de apareamiento con esta familia podrian exclarecer el significado de los patrones similares de alas en los tefrítidos.

The wings of tephritid fruit flies, often intricately patterned with spots, stripes, and blotches, are both lovely and mysterious. Within the superfamily Tephritoidea, only the wings of the Lonchaeidae are typically unmarked (Sivinski 2000), and in the Tephritidae, relatively few species, such as some *Bactrocera* spp. and *Neospilota* spp., have largely hyaline wings (e.g., Foote et al. 1993). Yet the significance(s) of these common and complex colorations is obscure.

In many tephritids, specialized wing movements occur in a sexual context (Sivinski et al. 2000). Wings are moved rapidly to create acoustic signals and perhaps to waft pheromones (e.g., Sivinski et al. 1984), but are also more slowly tilted and/or held away from the body in a variety of motions and postures (Headrick & Goeden 1994). These have been described as: (1) arching- the wings are held over the dorsum, slightly spread, and arched from the base to the apex such that the tips nearly touch the substrate; (2) enanation- the extension of both wings away from the body simultaneously; (3) hamation- the movement of the wings together over the dorsum or while they are extended away from the body; (4) lofting- both wings are extended upward 90 degrees above the substrate and supinated up to 90 degrees; and 5) supination- bringing the wing forward perpendicular to the long axis of the body while the ventral surface of the wing is turned to face anterior such that the costal margin of the wing is dorsal (White et al. 2000).

It is tempting to hypothesize that elaborate wing patterns and complex wing movements contribute to visual sexual signals (e.g., Bush 1969), and wing coloration, movements and mating systems frequently are correlated in Californian tephritid genera (Headrick & Goeden 1994). If patterns are sexual signals, it may be no coincidence that clear-winged lonchaeids are the only tephritoid family that appears frequently to mate in aerial swarms where wing patterns are unlikely to serve a communicative function (McAlpine & Munroe 1968; Sivinski 2000). However, there are several inconsistencies in the wing pattern and movement as sexual signal argument. Sexual dimorphisms might be predicted in courtship signals directed by males to females, but differences in visible-light wing patterns are relatively rare, although there are some striking exceptions. For example, *Aciurina idahoensis* Steyskal females have striped wings and males spotted, and in the related *A. semilucida* (Bates), female wings are striped and male wings fully infuscated (Headrick

& Goeden 2000). In some instances, e.g., *Trupanea* spp., dimorphisms are the opposite of expectation with male wings fainter or having fewer markings (Foote et al. 1993). Only the wings of two species, the Caribbean fruit fly, *Anastrepha suspensa* (Loew) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) have been examined for ultra-violet reflectance and transmittance (Sivinski et al. 2005). There is little UV transparency in the wings, and there is no sexual dimorphism when placed against a non-UV reflective background such as the leaves from which males are likely to be sexually signaling.

Other objections against patterned wings performing simple sexual signals include the complex wing movements of females, and the wing motions by males and females in apparently non-sexual situations. For example, in the genus *Goedenia* both sexes exhibit hamation throughout the day while grooming, resting, and feeding (e.g., Goeden 2002), although in such species there are often male wing movements unique to courtship (Headrick & Goeden 1994). In addition, markings are occasionally known to serve non-sexual purposes. When seen from behind, the wing patterns of *Zonostemata vittigera* (Coquillett) and *Rhagoletis zephyria* Snow create the illusion of a salticid spider seen face on and the resemblance deters spider attacks (Greene et al. 1987, Mather & Roitberg 1987).

In order to test the hypothesis that wing patterns have been sexually selected and contribute to sexual success, we first quantified the design of wing markings among North American Tephritdae and contrasted these markings to those of a distantly related brachyceran fly family, the Bombyliidae. The latter family was chosen for comparison and contrast because of the large number of species bearing wing patterns and its distant phylogenetic relationship to fruit flies. We then performed an experiment designed to test the importance of wing patterns in male *A. suspensa* sexual success.

Materials and Methods

The Nature and Distribution of Wing Patterns

We quantified wing marking patterns in the Tephritidae and Bombyliidae in the following manner. First, illustrations of wings were roughly divided into quadrants: frontal-distal, frontal-proximal, trailing-distal, and trailing-proximal. Then, the markings in each quadrant were characterized as either clear, dark, spotted, striped, or stellate (clear spots on a dark background) and given a numeric value depending on the patterns location on the wing (i.e., a lack of markings in the frontal-distal section would be given the numeric value of 1, in the frontal-proximal the same condition would be characterized as 6, in the trailing-distal it would be 11 and in the trailing-proximal 16). Wings were then categorized by the combined nature of the markings in each quadrant, e.g., dark in frontal proximal and clear in all others or striped in all quadrants but trailing-proximal. Thus a completely hyaline wing would be described by the combined numbers listed above and have the designation 161116.

The samples of wings were obtained from large taxonomic works (Tephritidae; Foote et al. 1993 and Bombyliidae; Hull 1973). The tephritid samples included a species from every North American genus (n = 57 in 3 subfamilies), and multiple species if there was diversity of wing pattern within the genus. Although we attempted to capture pattern diversity at the generic level, this method did not quantify the actual proportions of any particular pattern at the species level. For example, if genus X has 10 species, 9 of which have stripped wings and one spotted, both stripes and spots would be included in the data by a single example. The bombyliid sample contained for the most part single species from each of the 193 genera in 14 subfamilies, but multiple species were included when divergent wing patterns were apparent. However, we did not have access to the wing patterns of every species and as a result we were more likely to have underestimated the diversity in wing pattern in this family than in the Tephritidae. Because of the shortcomings in the samples, the results should be viewed as illustrating possible qualitative examples of convergence and divergence in wing patterns.

Sexual Success Following Wing Pattern Manipulation

The role of wing pattern in male sexual success was investigated by either obliterating or enhancing wing markings. First, virgin female *A. suspensa*, 15-21 d old, were transferred from 20 × 20 × 20-cm screen cages to smaller cylindrical screen cages (6.3 cm × 8.8 cm) prior to the experiment. Temperatures throughout the maturation and experimental periods were 25 ± 1°C and relative humidity 55% ± 5%. Three mature males 15-21 d old that had been treated in the three different manners described below were then added to the cage and their sexual successes noted. The three treatments were: (1) males removed from larger holding cage, chilled and then placed on a plastic sheet that had been stretched over ice; (2) dark wing markings on similarly treated males painted over with a brown India ink artists pen (Faber Castell, Pitt artist pen, medium point, brown, Cleveland, OH 44125); and (3) the hyaline spaces between dark wing markings filled in with the same ink. It was difficult to obtain a marking substance that would adhere to tephritid wings. India ink was the best of several alternatives, but even this coverage deteriorated rapidly over time. Because of this, males were marked the morning prior to sex-

ual exposure in the afternoon (during the last 4 h of the photoperiod). There were 100 replicates and the sexual successes of the various treatments were compared by contingency χ^2 test (Zar 1974).

Male characteristics other than wing pattern, specifically large male size, are known to influence sexual success (e.g., Sivinski et al. 1984). Because of this, we measured the wing lengths of all three males in each cage and they were given a relative rank. The summed ranks of successful males were then compared through a χ^2 test to an expected mean rank n replicates (expected product of rank = $2 \times 100 = 200$) had mating occurred regardless of size. There might also have been an interaction of pattern and size, so that a small male that suffered in competition with a larger rival overcame this disadvantage with a more attractive wing pattern. This possibility was examined with a Mann-Whitney nonparametric U-test (Zar 1974) by comparing the rank-sizes of mating males that had their patterns emphasized with ink and those whose patterns had been obliterated. Specifically, we looked to see if a male with one painted treatment was more likely to mate when smaller than was a male with the other treatment.

Results

The Nature and Distribution of Wing Patterns

Keeping in mind the differences in the tephritid and bombyliid samples, there are some suggestive similarities in the types of wing patterns and interesting differences in their purported distribution within their respective families (Fig. 1). Unmarked wings were more common in the Bombyliidae (36%), as were fully infuscated patterns. Pattern diversity appeared to be greater in the bombyliids with 42 patterns other than all hyaline displayed by 126 species (0.33 patterns / species) as opposed to 12 patterns in 61 species of Tephritidae (0.20 patterns / species). The majority of genus-level wing patterns in the Tephritidae were stellate or barred, with a smaller number of spotted and darkened-costal region patterns. Certain patterns were typical of different tephritid subfamilies: 87% of Trypetinae wings patterns could be characterized as barred, while the diversity in the Dacinae and Tephritinae was greater. The Dacinae is relatively species-poor in North America and excluded from further discussion. There were nine different wing patterns found in the Tephritinae, but the most common were stellate (29%) and, again, barred (36%).

Sexual Success Following Wing Pattern Manipulation

There were no significant differences in sexual success among wing treatments: Mated (untreated) = 37; (pattern enhanced) = 32; (pattern obliterated) = 31 ($\chi^2 = 0.62$; $P > 0.50$). There was no evidence that male size by itself played a role in male sexual success (mean size rank of successful males= 1.97; expected value from random mating= 2.0; $\chi^2 = 0.09$; $P > 0.95$). While males who mated and whose patterns had been enhanced tended to be relatively larger than males that mated and had their patterns obliterated (mean rank= 1.69 and 2.11, respectively), the difference was not significant ($U = 252$; $Z = 1.56$, $P = 0.12$).

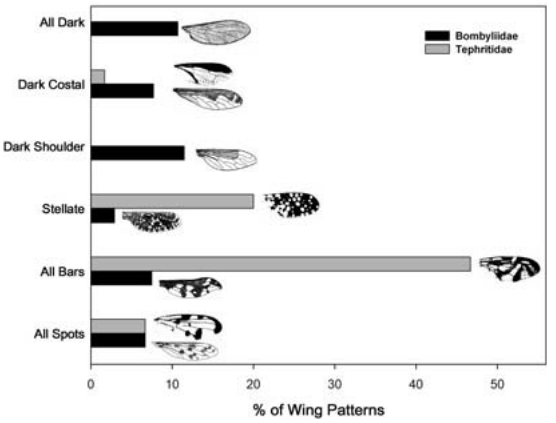

Fig. 1. The more common wing patterns found in Tephritidae (from Foote et al. 1993) and Bombyliidae (Hull 1973) and their proportions of the total number of pattered wings sampled.

Discussion

Given these results, wing markings remain lovely and mysterious; there was no evidence that markings played a role in the abilities of males to mate. However, we do not wish to overstate our results and conclude that markings have no communicative or sexual importance. Negative evidence is often difficult to interpret and, given the limitations of small cage experiments in the laboratory such as restricted movement and atypically high densities, and the likely inexact match of the brown ink to the color of the wing markings, a different experiment may well yield different results. That being said, the present result of finding no diminution of mating success following rather gross manipulation of the markings suggests that alternative explanations for the evolution of wing patterns in the Diptera should at least be considered (see True 2003).

One alternative, spider mimicry, was mentioned in the introduction. Also, the distinctive outline of an animal may be obscured by a disruptive pattern of stripes and spots (Cott 1940) and a resting fly with patterned wings might be thus camouflaged. Beside sexual signaling and adaptive coloration, another hypothesis is that pigments such as melanin are structural components of the wings and that any resulting visual effect is

fortuitous and without significance. For example, melanin pigmented surfaces warm up faster and cool down more quickly in a variety of insects, and melanic cuticle can be more resistant to abrasion than unpigmented cuticle (Majerus 1998). The numerous instances of coloration along the leading edge of the wing in the vicinity of the costal vein might be consistent with pigments strengthening a region that receives unusual stress. It may be that all of these factors play a role, and that ". . . wing displays and patterns are part of a dynamic system involving reproductive behavior, crypsis, and thermoregulation" (Headrick & Goeden 1994).

The seeming convergence in wing patterns between the Tephritidae and the Bombyliidae, at least in type if not frequency of design, might offer an opportunity for illuminating comparisons. Little has been published on the mating systems of bee flies. Males in *Comptosia* sp. near *latealis* Newman, perch in clearings and dart at nearby flying insects (Yeates & Dodson 1990). The wings in this genus are typically darkly pattered (Hull 1973), but male-male interactions occur in flight, as do at least some of the matings, which may argue against wing markings having any significance as courtship signals. Males of *Lordotus pulchrissimus* Williston form mating swarms (Toft 1989). The wings of this genus are generally hyaline (Hull 1973) and so are consistent with an aerial lack of signaling opportunity.

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References Cited

Bush, G. 1969. Mating behavior, host specificity, and the ecological significance of sibling species in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). Amerian Nat. 103: 669-672.

Cott, H. 1940. Adaptive Coloration in Animals. Methuen & Co., London.

Foote, R., F. Blanc, and A. Norrbom. 1993. Handbook of the Fruit Flies (Diptera: Tephritidae) of America North of Mexico. Comstock Publishing Associates, Ithaca, NY.

Goeden, R. 2002. Life history and descriptions of adults and immature stages of *Goedenia stenoparia* (Steyskal) (Diptera: Tephritidae) on *Gutierrezia californica* (De Candolle) Torrey and A, Gray and *Solidago californica* (Nuttal) (Asteraceae) in southern California. Proc. Entomol. Soc. Washington 104: 702-715.

Greene, E., L. Orsack, and D. Whitman. 1987. A tephritid fly mimics the territorial displays of its jumping spider predators. Science 236: 310-312.

Headrick, D., and R. Goeden. 1994. Reproductive behavior of California fruit flies and the classification and evolution of Tephritidae (Diptera) mating systems. Studia Dipterol. 1: 194-252.

Headrick, D., and R. Goeden. 2000. Behavior of flies in the subfamily Tephritinae, pp. 671-707 *In* M. Aluja and A. Norrbom [eds.] Fruit Flies (Tephritidae): Phylogeny and the Evolution of Behavior. CRC Press, Boca Raton, FL.

Hull, F. 1973. Bee flies of the World: the Genera of the Family Bombyliidae. Smithsonian Institution Press, Washington, D.C.

McAlpine, J., and D. Munroe. 1968. Swarming of loncaeid flies and other insects, with descriptions of new species of Lonchaeidae (Diptera). Canadian Entomol. 100: 1154-1178.

Majerus, M. 1998. Melanism: Evolution in Action. Oxford University Press. Oxford, UK

Mather, M., and B. Roitberg. 1987. A wolf in sheep's clothing: tephritid fruit flies mimic spider predators. Science 236: 308-310.

Sivinski, J. 2000. Breeding habits and sex in families closely related to the Tephritidae: opportunities for comparative studies of the evolution of fruit fly behavior, pp. 23-37 *In* M. Aluja and A. Norrbom [eds.], Fruit Flies (Tephritidae): Phylogeny and the Evolution of Behavior. CRC Press, Boca Raton, FL.

Sivinski, J., M., Aluja, G. Dodson, A. Freidberg, D. Headrick, K. Kaneshiro, and P. Landolt. 2000. Topics in the evolution of sexual behavior in the Tephritidae, pp. 751-792 *In* M. Aluja and A. Norrbom [eds.], Fruit Flies (Tephritidae): Phylogeny and the Evolution of Behavior. CRC Press, Boca Raton, FL.

Sivinski, J., T. Burk, and J. C. Webb. 1984. Acoustic courtship signals in the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Anim. Behav. 32: 1011-1016.

Sivinski, J., H. Klug, J. Shapiro, J. Lane, and R. Mankin. 2005. Ultraviolet reflectance on the heads and wings of *Anastrepha suspensa* (Loew) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Studia Dipterol. 11: 313-322.

Toft, C. 1989. Population structure and mating system of a desert bee fly (*Lordotus pulchrissimus*; Diptera: Bombyliidae). 1. Male demography and interactions. Oikos 54: 345-358.

True, J. 2003. Insect melanism: the molecules matter. Trends Ecol. and Evol. 18: 640-647.

White, I., D. Headrick, A. Norrbom, and L. Carroll. 2000. Glossar, pp. 881-924 *In* M. Aluja and A. Norrbom [eds.], Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL.

Yeates, D., and G. Dodson. 1990. The mating system of a bee fly (Diptera: Bombyliidae). I. non-resource-based hilltop territoriality and a resource-based alternative. J. Insect Behav. 3: 603-617.

Zar, J. 1974. Biostatistical Analysis. Prentice Hall Inc. Englewood Cliffs, NJ.

PARTITIONING NATIVE AND AUGMENTATIVE *TRICHOGRAMMA PLATNERI* (HYMENOPTERA: TRICHOGRAMMATIDAE) PARASITISM OF *AMORBIA CUNEANA* (LEPIDOPTERA: TORTRICIDAE) EGG MASSES IN SOUTHERN CALIFORNIA AVOCADO ORCHARDS

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The objective of this experiment was to test the efficacy of augmentative releases of *Trichogramma platneri* Nagarkatti against the avocado pest, *Amorbia cuneana* Walsingham, and to determine natural parasitism rates concurrently in the field by examining parasitism of sentinel *A. cuneana* egg masses placed in avocado orchards. The released wasps used in the experiments were marked with a unique phosphoglucose mutase (PGM) allele from *T. minutum* Riley that is absent in the coastal, native populations of the closely related *T. platneri* (Pinto et al. 1992). Wasps were produced by introducing the unique PGM marker into native *T. platneri* parasitoids by repeated backcrossing (>20 generations), to ensure that released lines were different only at the PGM locus. Wasps from each generation were electrophoresed for the unique PGM locus by the methods of Kazmer & Luck (1995) to ensure culture purity and quality assurance.

Studies were done in three blocks of 'Haas' avocado on ranches located at Temecula (Riverside Co.), Vista (San Diego Co.), and Moorpark (Ventura Co.), California. Orchard characteristics were quite variable between the Moorpark site and the Temecula/Vista sites. Temecula and Vista orchards were categorized as 'mature' and possessed trees that were at least 25 years in age, over 12.0 m in height and spaced between 7.5 and 10.5 m apart. In contrast the Moorpark orchard was categorized as 'immature' and possessed trees that were approximately 10 years old, less than 4.5 m in height, and spaced between 4.5 and 6.0 m apart. Parasitism and dispersal was evaluated by monitoring sentinel *A. cuneana* egg mass traps placed in 10 trees per orchard. Egg-masses were placed in nine avocado trees (30 egg masses per tree) arranged in the shape of a cross. Thus, there were two trees per arm of the cross and one tree at the center beneath which wasps were released. An additional tree located 10 rows east of the release tree containing egg masses served as a control. Within each tree, 15 egg-masses were arranged in the tree canopy 0.5-1.5 m above the ground and 15 egg-masses were placed 3.0-6.0 m above the ground in the tree canopy.

Egg-masses were placed in the ten experimental trees on day one. These egg masses were used to determine the amount of parasitism by resident *T. platneri* prior to release of the marked line. These egg-masses were replaced with fresh egg-masses on day four and followed immediately by the release of marked *T. platneri* in the center tree of each plot. The second egg-mass group was collected on day seven of the experiment and replaced with fresh egg-masses that were then collected on day 10 of the experiment. Thus, a total of 900 egg masses were placed in each orchard over a 10 d sampling period.

Approximately 40,522 wasps were released under the centrally located release tree. Egg-mass cards were collected and blackened eggs indicating parasitism were separated to collect parasitoids. Egg parasitism was calculated as the percentage of egg masses with at least one egg parasitized. Emerging parasitoids were snap frozen in liquid nitrogen and electrophoresed for PGM to determine if they were from the resident or released populations. Differences in the number of egg-masses parasitized between canopy treatments within trees, parasitism rate differences between adjacent and non-adjacent trees within an orchard, and comparisons between plot sites (mature vs. immature orchards) were analyzed by ANOVA (SAS Institute Inc. 1988).

Egg-masses placed in the experimental groves prior to release of the marked wasps remained unparasitized. Released wasps constituted almost all of the recorded parasitism as indicated by the presence of the unique PGM allele collected from wasps emerging from the sentinel egg-masses. A total of three sentinel egg-masses from a single tree at the Moorpark site were collected which contained wasps that had PGM alleles differing from those of the released *T. platneri*, however, they were similar to those of resident coastal *T. platneri* (Pinto et al. 1992), indicating that these wasps were the progeny of resident *T. platneri*. This represents only a small percentage (0.33%) of the observed parasitism in this plot. None of the 1,800 sentinel egg-masses placed in 10 trees over the three monitoring periods in either the Temecula or Vista plots showed *T. platneri* allozyme patterns typical of resident wasp populations.

Augmentative parasitism rates of the sentinel egg-masses in the upper and lower portions of the trees for all three locations were not significant ($F_{1,108} = 3.31$ $P > 0.05$). Parasitism rates of egg masses collected on day seven were significantly higher than those collected on day 10 for all three orchards studied with 78%, 89%, and 94% of the total parasitism observed occurring within three days of release for the Temecula, Vista, and Moorpark sites, respectively (Table 1). At each site,

TABLE 1. NUMBERS OF EGG MASSES PARASITIZED BY *TRICHOGRAMMA PLATNERI* BEFORE, DURING, AND AFTER RELEASE IN AVOCADO TREES IN THREE ORCHARDS.

Release Site	Treatment Pre Release	Release (eggs collected three days after release)	Post Release (eggs collected six days after release)
Vista	0/270 (0%)	71/270 (26.3%)	9/270* (3.3%)
Temecula	0/270 (0%)	64/270 (23.7%)	18/270* (6.6%)
Moorpark	0/270 (0%)	150/270 (55.6%)	10/270* (3.7%)

*Each row indicates significance at the 0.05 level for chi-square tests of independence between release and post release treatments for each of the three orchards.

parasitism rates between the outermost trees and trees adjacent to the release tree decreased significantly ($F_{1,108}$ = 41.02 $P < 0.05$). Thus, parasitoid searching efficiency appeared to be limited to the few trees in close proximity to the release tree and only for a brief period (<3 days) after release.

Parasitism rates appear to be affected by plant complexity and interplant distance based on this experiment as the Moorpark plot with smaller and closely spaced trees had higher levels of parasitism ($F_{2,108}$ = 11.79 $P < 0.05$). Larger, more complex trees may cause parasitoids to search more area per host encounter than in smaller less complex trees.

In conclusion, although indigenous *T. platneri* were extremely scarce, the use of unique allozymes incorporated into wasps released in the field may be an effective tool to accurately determine indigenous and augmentative parasitism rates concurrently. Moreover, augmentative releases are most effective three days after release and only in those trees immediately adjacent to parasitoid release points. Thus, point releases of *T. platneri* should not be spaced more than a few trees apart and should be performed every 3-4 days against *A. cuneana* in avocado.

SUMMARY

The use of a unique PGM allozyme marker was introduced into a culture of *Trichogramma platneri* used for augmentative field releases in an effort to distinguish between native and augmentative parasitism against *Amorbia cuneana* in avocado orchards. Although native parasitism rates were extremely low, the marker was useful in distinguishing native parasitoids from those released in the field. Augmentative releases were most effective up to three days post release and in those trees adjacent to the release trees. Orchard composition appears to affect parasitoid efficiency.

REFERENCES CITED

KAZMER, D. J., AND R. F. LUCK. 1995, Size fitness relationships in a field population of the egg parasitoid *Trichogramma pretiosum*: Ecol. 76: 412-425.

PINTO, J. D., KAZMER, D. J., G. R, PLATNER, AND C.A. SASSAMAN. 1992, Taxonomy of the *Trichgramma minutum* complex (Hymenoptera: Trichogrammatidae): allozymic variation and its relationship to reproductive and geographic data: Ann. Entomol. Soc. Am. 85: 413-422.

SAS INSTITUTE INC. 1988, SAS User's Guide: Statistics, Version 6 Edition. SAS Institute Inc., Cary, NC.

OCCURRENCE OF *LARRA BICOLOR* (HYMENOPTERA: SPHECIDAE), ECTOPARASITE OF MOLE CRICKETS (*SCAPTERISCUS* SPP.), IN COASTAL MISSISSIPPI

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Larra bicolor Fabricius (Hymenoptera: Sphecidae) is an immigrant species native to South America but introduced into Florida, Hawaii, and Puerto Rico for control of pest mole crickets (Frank et al. 1995; Frank & Sourakov 2002). *Larra* wasps are black with wings that are brown to blue with light-colored markings on the head. *Larra analis* Fabricius, which has a black abdomen with red typically at the tip, is native to Mississippi and the Gulf Coast region. *Larra bicolor*, in contrast, has a solid red abdomen (Frank & Sourakov 2002). The biology and ecology of this species has been reviewed recently (Frank et al. 199; Frank & Sourakov 2002) and will not be discussed here.

The first successful relocation of *L. bicolor* into North America was made between 1981-1983, when wasps were collected from Puerto Rico and released into five sites in Florida. From these releases, only wasps at the southernmost release site (Ft. Lauderdale) became established (Frank et al. 1995). Subsequent releases were made between 1988-1989 when presumably three species of wasps, *Larra bicolor*, *L. praedatrix*, and *L. godmani*, were collected from Bolivia and released along with parasitized hosts in three sites near Gainesville (Frank et al. 1995). There was no evidence that these releases were successful until *L. bicolor* was observed feeding on *Spermacoce verticillata* on the UF-Gainesville campus in 1993. Based on morphological characteristics of these wasps, they were presumably of Bolivian origin (Frank et al. 1995).

Larra bicolor has been released in Tifton GA and near Baton Rouge LA. Of these releases, only those released in GA have become established (W. Hudson & H. Frank, personal communication). Apart from these sites, Florida, Puerto Rico, and South America are the only other sources of *Larra bicolor*. This paper represents the first record of the natural expansion of *L. bicolor* outside of Florida, and the first record of this species in Mississippi.

On 29-IX-2004, hybrid bermudagrass (*C. dactylon* × *C. transvaalensis* 'Tifway') plots were being evaluated for mole cricket damage at Great Southern Golf Course, Gulfport, Harrison County, MS when a digger wasp resembling *Larra* was observed. This individual was not collected but its presence prompted a subsequent survey of damaged grass on that golf course. Three areas at Great Southern with fresh mole cricket damage were surveyed. Surveys were conducted by walking across mole cricket damaged areas of grass while looking for wasps resting on the turf. A soap solution, 30 ml of dishwashing soap per liter of water, was prepared in a 900-ml spray bottle with a trigger and used to collect wasps. When at rest on the turf, a wasp was shot with the soap solution repeatedly until dead, at which time it was collected and preserved in alcohol. Two digger wasps, *L. analis* and *L. bicolor*, were collected that day using this method. The University of Florida Insect Taxonomy Laboratory confirmed the identities of both species, and these were deposited as voucher specimens in the Mississippi Entomological Museum at Mississippi State University.

The same three damaged sites at Great Southern Golf Course were surveyed again on 1-X-2004. When a wasp was spotted, it was collected with the soap solution as before. Two *L. bicolor* were collected; of which one was exiting a mole cricket burrow. Two mole crickets also were observed moving across the grass. This is consistent with the previously described hunting behavior of *Larra* spp. (Frank & Sourakov 2002).

Because this was a first record of this species in Mississippi, it seemed important to determine whether *L. bicolor* was present on other golf courses in coastal Mississippi. On 11-X-2004, three additional courses in Harrison County were surveyed. The first, Bay Breeze Golf Course, also is located on the Mississippi Sound in Biloxi. Hybrid bermudagrass 'Tifway' tees, greens, and fairways that had fresh damage from mole crickets were surveyed for *L. bicolor* as previously described. Five wasps were observed on four different holes, but only three, all *L. bicolor*, were collected.

The second course surveyed that day was the President-Broadwater Golf Course in Biloxi. This course had abundant damage from mole crickets, but no wasps were observed or collected. Unlike Bay Breeze and Great Southern, this course had no naturalized areas where blooming wildflowers were present, nor were there any plantings of blooming woody or herbaceous ornamentals. The last course in Harrison County, Sunkist Country Club, had only one area with mole cricket damage and no *Larra* were observed or collected.

On 12-X-2004, the Bridges Golf Course at the Casino Magic resort in Bay St. Louis, Hancock County, MS was surveyed. This course was chosen because it borders the Mississippi Sound and has

abundant naturalized areas of wildflowers. Areas, primarily greens and tees, on each hole had some mole cricket damage. No *Larra* were observed or collected.

The last site surveyed was St. Andrews Golf Course, Ocean Springs, Jackson County, MS on 18-X-2004. This course is located on the Mississippi Sound and has a perennial mole cricket population. The three areas surveyed were on close roughs of Bermuda grass near tees or greens, and all had extensive damage caused by mole crickets. One *L. bicolor* was collected from this course.

The results of these surveys indicate that while *L. bicolor* is present in coastal Mississippi, it is not abundant. Only those courses that were adjacent to the Mississippi Sound had *L. bicolor*. Perhaps these sites, being buffered by the coastal waters, provide a suitable microclimate where this wasp can successfully over winter.

I thank the staff of Great Southern Golf Course, Sunkist Country Club, Bay Breeze Golf Club, President Golf Course, St. Andrews Golf Course, and The Bridges at Casino Magic for cooperation with the survey. I thank Lionel Stange, Lyle Buss, and Howard Frank for identification of collected specimens. Richard Brown, Linda Andrews, and Jianzhong Sun (MS State University) provided helpful comments on an earlier draft of this manuscript. This paper is No. J10668 of the Mississippi State Agricultural Experiment Station.

Summary

Larra bicolor (Hymenoptera: Sphecidae) is an ectoparasite of exotic mole crickets (*Scapteriscus* spp., Orthoptera: Gryllotalpidae). This wasp was introduced into Florida as a biological control agent, but natural spread of this insect has not been reported outside of that state. In 2004, specimens of *Larra bicolor* were collected from three golf course sites in coastal Mississippi. This find is the first record of this species in Mississippi and represents the first record of natural movement of this wasp outside of Florida.

References Cited

Frank, J. H., and A. Sourakov. 2002. Larra wasps; mole cricket hunters. University of Florida pub. No. EENY-268. http://creatures.ifas.ufl.edu/beneficial/Larra_wasps.htm

Frank, J. H., J. P. Parkman, and F. D. Bennett. 1995. *Larra bicolor* (Hymenoptera: Sphecidae), a biological control agent of *Scapteriscus* mole crickets (Orthoptera: Gryllotalpidae), established in Northern Florida. Florida Entomol. 78: 619-623.

ENHANCED EGG LAYING IN ADULT PREDATORS FED ARTIFICIAL DIET SUPPLEMENTED WITH AN EMBYONIC CELL LINE DERIVED FROM EGGS OF *EPHESTIA KUEHNIELLA* ZELLER (LEPIDOPTERA: PYRALIDAE)

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Suboptimal fecundity in entomophagous insects reared on artificial diet is a common problem and barrier in implementing cost-effective large-scale production of beneficial insects for augmentative biological control (Grenier et al. 1994). The artificial diets of a number of entomophagous insects can only be improved by adding insect tissues such as hemolymph to the diet (Nettles 1990). The use of insect materials, however, is not feasible because of the labor required and associated problems (e.g., melanization), and other means of improving the diets are needed. One obvious approach is to identify the compounds in insect materials that are responsible for improving fecundity, however, this is difficult and none have been identified yet (Ferkovich & Shapiro 2004a). Another approach is to use insect cells to improve artificial diets for predators. Insect cell lines have been tested on several parasitoids with promising results (Rotundo et al. 1988; Ferkovich et al. 1994; Hu et al. 1999; Heslin et al. 2005). The fecundity of the insidious flower bug, *Orius insidiosus* (Say), reared on artificial diet was improved with IPLB-PiE, a cell line derived from eggs of the Indian meal moth, *Plodia interpunctella* (Hübner) (Ferkovich & Shapiro 2004b). Because insectaries generally produce the predator on eggs of the Mediterranean flower moth, *Ephestia kuehniella* Zeller (Association of Natural Bio-control Producers), a cell line (Ek-x4V) was recently developed from eggs of *E. kuehniella* (Lynn & Ferkovich 2004). In this study, we investigated the potential of using the *Ephestia* cell line (Ek-x4V) to promote egg production of adults of *O. insidiosus* maintained on artificial diet.

The IPLB-PiE and the Ek-x4V cell lines were cultured as described earlier (Lynn 1996; Lynn & Ferkovich 2004). Briefly, the IPLBPiE cells were grown in modified TNM-FH insect medium (Sigma, St. Louis, MO) in 25-cm^2 culture flasks for 7 days. For larger-scale culture of the cell lines, the PiE cells were grown in 250 ml of medium in 500-ml magnetic spinner flasks (Bellco Glass, Vineland, NJ) at 24.9E for 14 days. The Ek-x4V cells were grown in SF900II medium (Invitrogen Corp., Grand Island, NY) in 25-cm^2 culture flasks for 14 days but could not be cultured in the larger spinner flasks because they grew as cellular aggregates and the spinning motion of the flasks interfered with their growth. Both cell lines were centrifuged (1370 *g* for 3 min) in graduated conical glass tubes to obtain a pellet of cells. The pellets were resuspended in distilled water and washed 2×. Cells from each line were then bioassayed in two tests.

The objective of the first bioassay was to determine if the Ek-x4V cells would affect the oviposition rate of females in a dose-response manner. Washed cells were centrifuged to obtain 0.25, 0.5, 0.75, and 1.0 ml of soft pellets of cells which were each homogenized with a hand-held homogenizer. Aliquots (20µ1) of the homogenates were removed and assayed for protein by the Lowry procedure (Protein Assay Kit, Sigma, St. Louis, MO). Diet ingredients (0.33 g brewers yeast, 0.03 g sucrose, 0.18 g soy protein acid hydrolysate, 3.8 mg of 99% palmitic acid (all from Sigma, St Louis, MO), 0.04 g fresh chicken egg yolk, and 0.08 g honey) were added to each of the tubes to give a final volume of 1.2 ml. The diet was then encapsulated in Parafilm© (25µl capsules) and bioassayed as described earlier (Ferkovich & Shapiro 2004b). Adults (three days after eclosion) were fed 3 mg of *E. kuehniella* eggs (Eph Eggs), two capsules of artificial diet (AD), and two capsules of artificial diet + Ek cells (Ek Cells) for six days. Each treatment diet consisted of six females and four males, two Parafilm© capsules of water (25µl each) and two capsules (25µl each) of treatment diet with four replicates per treatment. Diets were replaced daily and mortality was recorded. At the end of the sixth day, a green bean pod was placed in each jar as an oviposition substrate and the number of eggs oviposited during a 24-h period were recorded.

The objective of the second bioassay was to compare artificial diet fortified with Ek-x4V cells (Ek Cells) against diet fortified with the IPLB-PiE cells (PiE Cells). Cells (0.74 ml, 52 mg protein) from each cell line were added to diet. Artificial diet (AD) and *Ephestia* eggs (Eph Eggs) treatments were also included in the bioassay.

Data were analyzed by one-way ANOVA with Dunnet's test for comparison of treatment means with control and Newman-Keuls post test for multiple mean comparisons (GraphPad Software, San Diego, CA).

Females fed on the Ek Cells diet oviposited significantly more eggs at the 0.75 ml-, and 1.0- ml doses of cells per 1.2 ml of diet than those that fed on the AD ($F = 3.8, df = 4, P = 0.02$) (Fig. 1). In comparing the PiE Cells diet with the Ek Cells diet, egg production on both diets approached that of females fed Eph Eggs and both diets significantly increased the average rate of oviposition relative

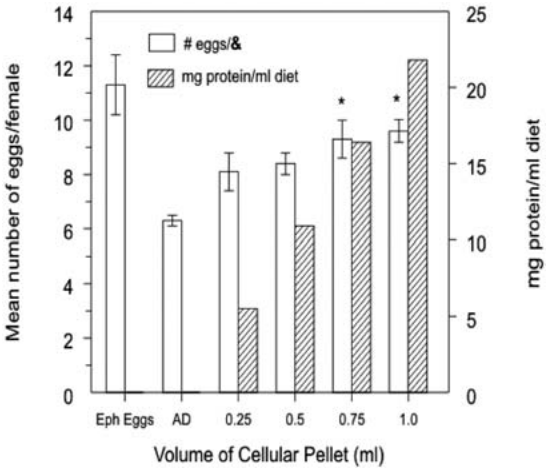

Fig. 1. Comparison of mean oviposition by females fed whole *Ephestia* eggs (Eph Eggs), artificial diet (AD) and artificial diet supplemented with aliquots of Ek-x4V h cells per 1.2 ml of diet. Dunnet's test was used to compare the treatment means against the artificial diet; asterisk indicates that the treatment means are significantly different from the artificial diet, ($P < 0.05$); error bars = standard error.

to the control AD. Neither of the cell line-supplemented diets, however, was better than the other in improving egg production (Fig. 2). The IPLB-PiE cell line is easier to culture since it grows as a suspension and lends itself to culture in spinner flasks and higher densities of cells can be achieved in less time. In contrast, the Ek-x4V line grows as aggregates of cells composed of organized vesicles that did not grow well in spinner flasks to attain the same level of cell densities as the PiE line during the 14 day culture period.

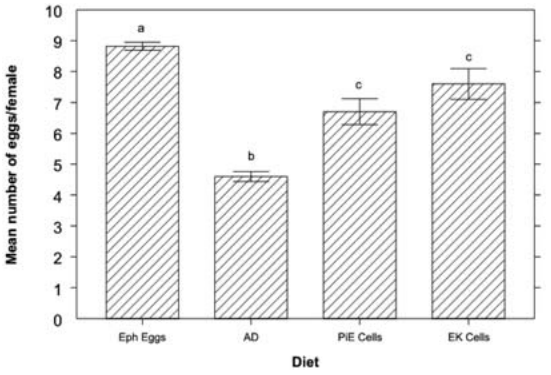

Fig. 2. Comparison of mean oviposition by females fed whole *Ephestia* eggs (Eph Eggs), artificial diet (AD), artificial diet + Pie cells (PiE Cells), and artificial diet + Ek cells (Ek Cells); bars with the same letter are not significantly different (Newman-Keuls method, $P > 0.05$); error bars = standard error.

Consequently, our present findings do not indicate that the EK-x4V cell line affords an advantage over the IPLB-PiE cell line as a diet supplement for improving the fecundity of *O. insidiosus*. The PiE cell line appears to be a better candidate for future studies directed at adapting the PiE line to grow in cheaper, serum-free cell culture medium for use in a large scale fermentation system and methods of preserving and packaging the cells for evaluation in artificial diets by insectaries.

We thank Drs. Terry Arbogast and Simon Yu for helpful comments. We also appreciate the excellent technical assistance in this study of Delaine Miller.

Summary

Artificial diet supplemented with the Ek-x4V cell line significantly enhanced the average rate of oviposition relative to the control diet but was not better than diet augmented with the IPLB-PiE cell line. The Ek-x4V line grew as aggregates of hollow vesicles of cells in contrast to the IPBL-PiE line which grew as a suspension of unattached cells and did not produce sufficient cell growth in spinner flasks for large-scale production of the cells. Therefore, the EK-x4V cell line does not afford an advantage over the IPLB-PiE cell line as a diet supplement for improving the fecunduty of *O. insidiosus*.

Literature Cited

Association of Natural Bio-control Producers. 2001. Supplier members, *www.anbp.org,* Santa Ana, CA, 92705.

Ferkovich, S. M., and J. Shapiro. 2004a. Comparison of prey-derived and non-insect supplements on egg-laying of *Orius insidiosus* (Hemiptera: Anthocoridae) maintained on artificial diet as adults. Biol. Control 31: 57-64.

Ferkovich, S. M., and J. Shapiro. 2004b. Increased egg-laying in *Orius insidiosus* (Hemiptera: Anthocoridae) fed artificial diet supplemented with an embryonic cell line. Biol. Control. 31: 11-15.

Ferkovich, S. M., H. Oberlander, C. Dillard, and E. Leach. 1994. Embryonic development of an endoparasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae) in cell line-conditioned media. In Vitro Cell Dev. Biol. 30A: 279-282.

Heslin, L. M., R. A. Kopittke, and D. J. Merritt. 2005. The role of insect cell lines in an artificial diet for the parasitoid wasp, *Trichogramma pretiosum*. Biol. Control 33: 186-193.

Grenier, S., P. D. Greany, and A. C. Cohen. 1994. Potential for mass release of insect parasitoids and predators through development of artificial culture techniques, pp. 181-205 *In* D. Rosen, F. D. Bennett, and J. L. Capinera [eds.], Pest Management in the Subtropics: Biological Control—a Florida Perspective. Intercept Publishers, Andover, Hampshire, England.

Hu, J. S., D. B. Gelman, R. A. Bell, and D. E. Lynn. 1999. *In vitro* rearing of *Edovum puttleri*, an egg parasitoid of the Colorado potato beetle, on artificial

diets: effects of insect cell line-conditioned medium. Arch Insect Biochem Physiol. 40: 173-182.

LYNN, D. E. 1996. Development and characterization of insect cell lines. Cytotechnology 20: 3-11.

LYNN, D. E., AND S. M. FERKOVICH. 2004. New cell lines from *Ephestia kuehniella*: characterization and susceptibility to baculoviruses, 5 pp. Journal of Insect Science 4:9. Available online: insectscience.org/4.9.

NETTLES, W. C. 1990. *In vitro* rearing of parasitoids: Role of host factors in nutrition. Arch. Insect Biochem. Physiol. 13: 167-175.

ROTUNDO, G., R. CAVALLORO, AND E. TREMBLAY. 1988. *In vitro* rearing of *Lysiphlebus fabarum* (Hym.: Braconidae). Entomophaga 33: 264-267.

IN VITRO PRODUCTION OF HYPHAE OF THE GRASHOPPER PATHOGEN *ENTOMOPHAGA GRYLLI* (ZYGOMYCOTA: ENTOMOPHTHORALES): POTENTIAL FOR PRODUCTION OF CONIDIA

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Fungi of the *Entomophaga grylli* pathotype or species complex (Zygomycota: Entomophthorales, Entomophthoraceae) are ecologically obligate parasites of grasshoppers and locusts (Orthoptera); their host range includes many economically important orthopteran species worldwide (Carruthers et al. 1994; Bidochka et al. 1995). The species of the *E. grylli* complex (heretofore collectively called *E. grylli*) are highly pathogenic and can cause spectacular field epizootics. They have attracted interest as insect control agents, in both classical and augmentative biological control of Orthoptera (Carruthers et al. 1994; Sawyer et al 1994; Bidochka et al. 1995). Penetration through insect cuticle by germinating conidia is the universal invasion route of entomopathogenic fungi, including *E. grylli*. In the *E. grylli* species complex, no production of walled cells (hyphae or conidia) has been reported to occur on the few artificial media that support its stable growth. These conidia must be produced on hyphal conidiophores.

Upon landing on a host, *E. grylli* conidia can penetrate the cuticle with germ tubes and reach the hemocoel; once there, the fungus produces amoeboid protoplasts. These fragile, wall-less, amoeboid vegetative cells are the invasive phase in the orthopteran haemocoel (Ramoska et al. 1988; Carruthers et al. 1994). The wall-less condition of protoplasts seems to allow them to remain undetected and escape the insect's immune system (Roberts & Humber 1982). After proliferating and killing the host, protoplasts produce cell walls. This results, in some members of the *E. grylli* species complex, in walled hyphae that emerge through the host cuticle and produce conidiophores and infective conidia externally on the insect. A latent intermediate stage (resting spores) inside the host can also result after production of cell walls by protoplasts. External, aerial spores (conidia), the only infective stage in nature, are responsible for the often-rapid horizontal transmission leading to epizootics (Carruthers et al. 1994).

Unfortunately, *E. grylli* is fastidious regarding its artificial culture; these fungi will rarely grow on solid media. In practice, they can only be propagated either *in vivo* in their orthopteran hosts, or in complex liquid media such as Graces's insect tissue culture medium (Ramoska et al. 1988; Bidochka et al. 1995). Further, in Grace's and similar media these fungi have so far been reported to grow only as protoplasts, not hyphae. Protoplasts cannot be distributed directly as a biological control tool. Their lack of cell wall renders them very fragile, and they are neither infective upon application to the insect's cuticle nor upon ingestion. Thus, manipulation of *E. grylli* usually requires that considerable amounts of appropriate live insect hosts are available.

Entomophaga grylli protoplasts are infective upon injection in their hosts, and they induce typical lethal mycoses. In classical biological control projects, live, protoplast-injected orthopterans have been released into populations free of these diseases (Carruthers et al. 1994; Bidochka et al. 1995; Sánchez Peña et al.1996). Production of *E. grylli* infective conidia on artificial media would facilitate its use as a bioinsecticide, analogous to other entomopathogenic fungi. Manipulation of fungi in biocontrol is possible if stable, resistant walled cells (such as infective conidia, or at least hyphae) can be produced. Hyphae of entomopathogenic fungi such as *Beauveria*, *Hirsutella,* and *Metarhizium* spp. (Deuteromycota: Hyphomycetes), and *Zoophthora* and *Pandora* (Zygomycota: Entomophthorales) can be massively produced in artificial media. These hyphae subsequently produce infective walled cells (conidia) that cause lethal disease in insects in the field (Rombach et al. 1986; Wraight et al. 1986; Sánchez-Peña and Thorvilson 1991; McCoy 1996).

In the *E. grylli* species complex, no production of walled cells (hyphae or conidia) has been reported to occur on the few artificial media that support its stable growth (i.e., Humber & Hansen 2004). Herein I report the observation of the *in vitro* transition from protoplasts to walled, myceliar (hyphal) vegetative phase in *E. grylli* pathotype I.

Entomophaga grylli pathotype I (informally called *E. macleodii*) was isolated from *Camnula pellucida* collected in Alpine, Arizona, passed through *Melanoplus bivittatus* (Orthoptera: Acrididae), and reisolated in Grace's insect tissue culture medium plus 1% fetal bovine calf serum (FBS) (Invitrogen-GIBCO, Carlsbad, CA) and stored in liquid nitrogen for 13 years as strain ARSEF 770 (Humber & Hansen 2004). The author passed ARSEF 770 through *Camnula pellucida* and reisolated it, originating ARSEF strains 4948, 4949, 4950, 4952, and 4953 (strains deposited at PPRU, USDA, ARS, Ithaca, NY). All *in vitro* cul-

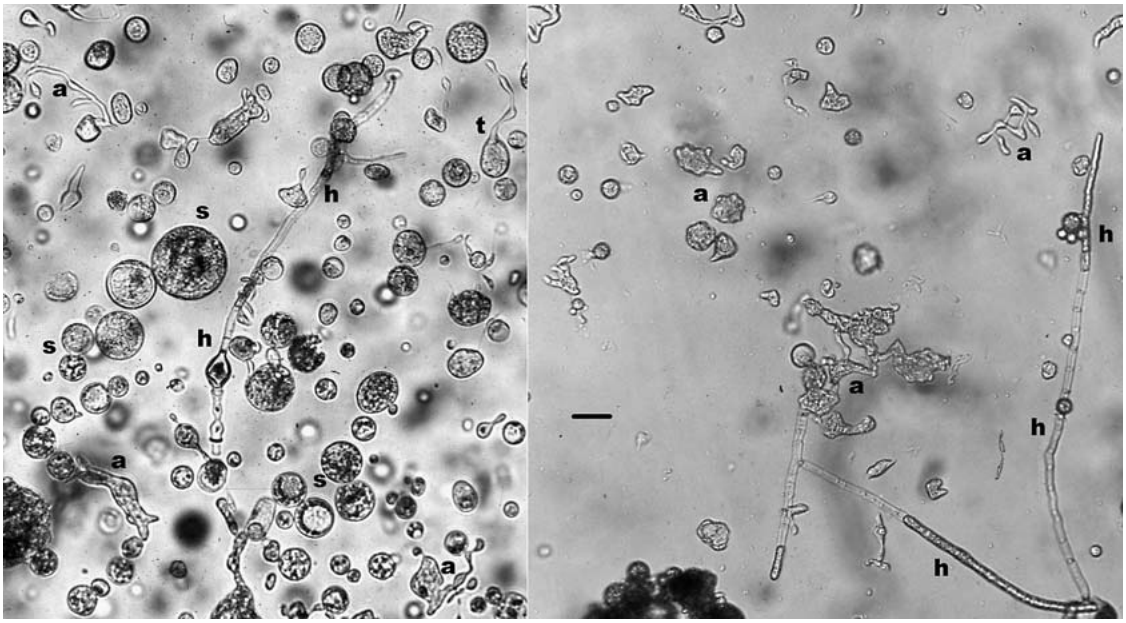

Fig. 1. *Entomophaga grylli* pathotype 1 cells from in vitro liquid culture: a, amoeboid and moniliform, motile protoplasts; s, spherical protoplasts; t, transition between amoeboid and spherical protoplasts; h, walled, septate hyphae. Notice typical entomophthoralean hyphae with apical cytoplasm and empty, septated spaced behind the tip. Bar = 20 microns.

tures were grown in 8-10 ml of Graces's medium plus FBS as described, in vented tissue culture flasks (9 × 5 × 2.5 cm) (TPP-MIDSCI, St. Louis, MO), under diffuse fluorescent and natural light.

After at least four weeks on artificial medium, numerous irregularly shaped, amoeboid as well as spherical protoplasts were observed in flasks from all these isolates (Fig. 1). Protoplasts are the propagules normally observed in such cultures of *E. grylli*. Some of the protoplasts assumed filamentous growth as walled hyphae. Hyphal growth was observed originating from both amoeboid and spherical protoplasts (Fig. 1 and 2). As described, *E. grylli* pathotype I changes from protoplasts to hyphae in its hosts. The hyphae then can produce the infective units, conidia, on conidiophores externally on the insect's surface. The transitions reported herein show that the first part of development leading to production of infective conidia (i.e., transition from protoplasts to hyphae) can be obtained in artificial media. It is possible that further differentiation to conidia can be obtained from artificial medium-grown biomass. This would facilitate the deployment of *E. grylli* fungi as biological control tools of Orthoptera. I expect that this report will stimulate searches towards completing the life cycle of these fungi *in vitro*, and towards the production of conidia from fungal biomass grown in artificial media.

The logistic support of R. Carruthers, J. Correa, M-L Cummings, and S. P. Wraight is acknowledged.

Summary

The species of the *Entomophaga grylli* complex that are fastidious grasshopper and locust pathogens have not been reported to complete their life

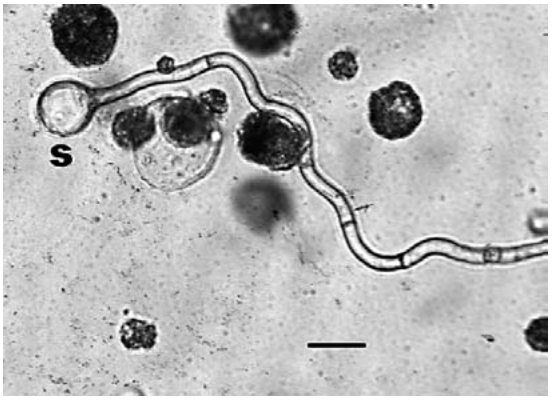

Fig. 2. *Entomophaga grylli* pathotype 1 walled, septate, empty hypha originating from now empty spherical cell. Bar = 35 microns.

cycles *in vitro*. The production of true hyphae in semisynthetic, liquid medium is described for *E. grylli* pathotype 1, a pathogen of banded-winged grasshoppers (Orthoptera: Oedipodinae). It is possible that induction of further differentiation of *E. grylli* hyphae grown on artificial medium might lead to production of infective conidia for biocontrol of pest Orthoptera.

REFERENCES CITED

BIDOCHKA, M. J., S. R., WALSH, M. E. RAMOS, R. J. ST. LEGER, J. C. SILVER, AND D. W. ROBERTS. 1995. Fate of biological control introductions: Monitoring an Australian fungal pathogen of grasshoppers in North America. Proc. Nat. Acad. Sci. USA, 93 (2), 918-921.

CARRUTHERS, R. I., M. E. RAMOS, T. S. LARKIN, D. L. HOSTETTER, AND R. S. SOPER. 1997. The *Entomophaga grylli* (Fresenius) Batko species complex: Its biology, ecology, and use for biological control of pest grasshoppers. Memoirs Can. Entomol. Soc 171: 329-353.

HUMBER, R. A., AND K. S. HANSEN. 2004. Catalog of Strains. Plant Protection Research Unit, USDA-ARS, Ithaca, New York.

MCCOY, C. W. 1996. Pathogens of eriophyoids, pp. 481-490 *In* E. E. Lindquist, M. W. Sabelis, and J. Bruin [eds.], Eriophyoid Mites-Their Biology, Natural Enemies and Control. Elsevier Science B.V., Amsterdam.

RAMOSKA, W. A., A. E. HAJEK, M. E. RAMOS, AND R. S. SOPER. 1988. Infection of grasshoppers (Orthoptera: Acrididae) by members of the *Entomophaga grylli* species complex (Zygomycetes: Entomopthorales). J. Invertebr. Pathol. 52: 309-313.

ROBERTS, D. W., AND R. A. HUMBER. 1984. Entomopathogenic fungi, *In* D. W. Roberts, and J. R Aist [eds], Infection Processes of Fungi. Rockefeller Foundation. New York.

ROMBACH, M. C., R. M. AGUDA., B. M SHEPARD, AND D. W. ROBERTS. 1986. Entomopathogenic fungi (Deuteromycotina) in the control of the black bug of rice, *Scotinophara coarctata* (Hemiptera; Pentatomidae). J. Invertebr. Pathol. 48: 174-179.

SÁNCHEZ-PEÑA, S. R., R. CARRUTHERS, M-L. CUMMINS, J. CORREA, AND S. WRAIGHT. 1996. Entomopatógenos como agentes de control biológico clásico: experiencias con *Entomophaga grylli* en el control biológico de saltamontes y langostas. Memorias XIX Congreso, Sociedad Mexicana de Control Biológico, Culiacán, Sinaloa. p. 8-10.

SÁNCHEZ PEÑA, S. R., AND H. G. THORVILSON. 1991. The entomopathogenic fungus *Beauveria bassiana* for fire ant control in nursery stock, pp. 94-105 *In* M. Mispagel [ed.], Proc. 1991 Imported Fire Ant Conference, U. of Georgia, Atlanta, GA.

SAWYER, A. J., M. E. RAMOS, T. J. POPRAWSKI, R. S. SOPER, AND R. I. CARRUTHERS. 1997. Seasonal patterns of cadaver persistence and sporulation by the fungal pathogen *Entomophaga grylli* (Entomophthorales: Entomophthoraceae) infecting *Camnula Pellucida* (Orthoptera: Acrididae). Memoirs Can. Entomol. Soc. 171: 835-855.

WRAIGHT, S. P., S. GALAINI-WRAIGHT, R. I. CARRUTHERS, AND D. W. ROBERTS. 1986. Field transmission of *Erynia radicans* to *Empoasca* leafhoppers in alfalfa following application of a dry, mycelial preparation, p. 223 *In* R. A. Samson, J. M. Vlak, and D. Peters [eds.], Fundamental and Applied Aspects of Invertebrate Pathology. Proc. IV Int. Colloq. of Invertebrate Pathology. Wageninen, The Netherlands. 560 pp.

A COMPARISON OF FECAL PROTEIN CONTENT IN MALE AND FEMALE CAT FLEAS, *CTENOCEPHALIDES FELIS* (BOUCHE´) (SIPHONAPTERA: PULICIDAE)

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The diet of the larval cat flea, *Ctenocephalides felis* (Bouche´), has been the focus of study by a number of researchers (Bruce 1948; Hsu et al. 2002; Moser et al. 1991; Richman et al. 1999). The consensus is that adult flea feces are the essential nutritional requirement for developing cat flea larvae. Various other organic materials found within the micro-habitat of larvae, previously thought to be of importance, have proven to have no significance in the diet (Strenger 1973).

There is an incomplete utilization of host blood imbibed by adult cat fleas (Hinkle et al. 1991; Silverman & Appel 1994). Hinkle et al. (1991) reported that the protein content of cat flea feces was actually higher than the bovine blood upon which they fed, while Silverman & Appel (1994) found only a slight difference. The fact that adult flea feces are nutritionally necessary for larval development has led to the suggestion that there may be a unique form of parental investment exhibited in cat fleas (Hinkle et al. 1991; Silverman & Appel 1994).

The objectives of this study were to compare protein content in the feces of adult male and female *C. felis* over a 10-day feeding period and to examine the extent to which male fleas may provide protein for developing flea larvae.

Adult male and female cat fleas were reared from eggs at the Missouri Research Center Laboratory in Fulton, Missouri. Fleas were held in an incubator at 28°C and 85% RH and exposed to a 12:12 light/dark regime until used for study purposes.

At 25 days following the egg collection date, fleas were sorted to sex on a vacuum stage under a stereomicroscope for identification. Males and females were aspirated from the stage separately and placed individually into test tubes.

A total of 190 female and 285 male fleas were fed bovine blood containing a 20% solution of sodium citrate in an artificial membrane system (FleaData, Inc., Freeville, NY) similar to the one described by Wade & Georgi (1988). Blood was obtained from a Holstein calf that had no history of exposure to parasiticides. Blood in the feeding sleeves was maintained at approximately 38°C to simulate blood temperature of the live host. Fleas were provided with fresh blood daily from a container refrigerated at 4°C. After six days, fresh blood was obtained from the same calf.

Flea feeding cages were 6 mm in diameter and 1.5 mm deep and consisted of two chambers. The upper chamber containing fleas had a fine nylon mesh top through which fleas imbibed the blood and a bottom of coarse mesh that allowed feces to fall through but which also prevented fleas from escaping. A very thin layer of clean cat hair was placed in the upper chamber with the fleas to facilitate movement onto the feeding screen. The lower chamber was fastened to the upper chamber but was removable. The lower chamber had a very fine mesh bottom, and its sole purpose was to collect feces that fell from fleas in the upper chamber. Every 24 h for a 10-day period, the lower chamber was removed from each feeding cage and the feces were transferred to ½-dram glass vials. Flea feeding cages were shaken vigorously before removal of the lower chamber to make sure all feces were captured for each particular day. All vials were immediately placed in a freezer at –20°C after collection.

One milligram aliquots of adult male and female feces from each of 10 consecutive feeding days were dissolved in 1 ml of de-ionized water, vortexed, and centrifuged for five minutes at 3000 rpm to move any foreign material to the bottom of the test tube. Thirty microliters of supernatant from each sample were combined with 1.5 ml Coomassie Blue reagent (Pierce) in a modified Bradford (1976) total protein assay. Samples were vortexed again and then transferred to square disposable 10-mm cuvettes (Elkay Products) where they were allowed to incubate at room temperature for 10 minutes.

Six replicate samples from each of the 10 days for male and female fleas were measured by absorbance for total protein content with a spectrophotometer (Shimadzu UV-1601) at a wavelength of 595 nm. Protein concentrations were estimated with reference to absorbance values obtained for a series of standard protein dilutions of known concentration, which were assayed along with the flea fecal samples.

A standard curve was prepared by plotting the average blank-corrected 595 nm measurement for each standard versus its concentration in (μg/mg). Total fecal protein concentrations for male and female fleas on each of the 10 days was analyzed with a t-test ($\alpha = 0.05$).

In comparing total protein concentration between adult male and female feces over a 10-day period, a significant difference was found only on day 1 of the study with males having a higher total protein concentration (Table 1). The mean to-

TABLE 1. MEAN ± SE PROTEIN CONCENTRATION (µG/MG) MEASURED DAILY FROM C. FELIS DRY MALE AND FEMALE FECES.

Sex	Day 1	2	3	4	5	6	7	8	9	10
Male	2979.8 ± 123.4*	2639.8 ± 94.1	2633.2 ± 93.7	2635.8 ± 96.1	2561.2 ± 87.6	2621.8 ± 98.6	2647.0 ± 107.7	2479.5 ± 30.7	2646.0 ± 92.8	2598.2 ± 107.6
Female	2597.8 ± 27.4*	2613.3 ± 101.7	2507.5 ± 34.59	2483.0 ± 26.0	2559.2 ± 70.9	2656.2 ± 89.7	2647.0 ± 99.3	2634.2 ± 81.0	2530.5 ± 27.4	2622.2 ± 105.2

Means on the same day followed by an asterisk are significantly different ($\alpha = 0.05$).

tal protein concentration between males and females for the other 9 days of the feeding period resulted in no significant difference between the two sexes.

SUMMARY

This study determined that male feces contain the same amount of protein as female feces when measured as total protein. Protein content in male feces was as high as, or significantly higher than, female feces throughout the entire period of this study. These results demonstrate that male *C. felis* are equally capable of providing protein for developing larvae, as are females.

Whether or not the inefficient use of host blood warrants a form of parental investment is debatable. An alternate hypothesis is that adult fleas are imbibing large volumes of blood to glean nutrients that may be at low levels in the blood. A comparison of a wider range of host blood nutrients to the feces excreted could support such a hypothesis.

The significance of the differences in fecal protein content observed on day 1 can only be conjectured without further investigation. While the data presented here indicate that there is equal qualitative investment between the sexes, the greater volume of feces produced by the female may be indicative of a greater quantitative investment.

In conclusion, these data demonstrate that male *C. felis*, while providing a smaller volume of food, are providing a food source as equally rich in total protein as the female of the species.

ACKNOWLEDGMENTS

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REFERENCES CITED

BRADFORD, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein binding. Analytical Biochem. 72, 248-254.

BRUCE, W. N. 1948. Studies on the biological requirements of the cat flea. Ann. Entomol. Soc. Amer. 41: 345-352.

HINKLE, N. C., P. G. KOEHLER, AND W. H. KERN. 1991. Hematophagous strategies of the cat flea (Siphonaptera: Pulicidae). Florida Entomol. 74(3): 377-385.

HSU, M. H., Y. C. HSU, AND W. J. WU. 2002. Consumption of flea feces and eggs by larvae of the cat flea, *Ctenocephalides felis*. Medical and Veterinary Entomol. 16: 445-447.

MOSER, B. A., P. G. KOEHLER, AND R. S. PATTERSON. 1991. Effect of larval diet on cat flea (Siphonaptera:

Pulicidae) development times and adult emergence. J. Econ. Entomol. 84(4): 1257-1261.

RICHMAN, D. L., P. G. KOEHLER, AND R. J. BRENNER. 1999. Spray-dried bovine blood: An effective laboratory diet for *Ctenocephalides felis felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 36(3): 219-221.

SILVERMAN, J., AND A. G. APPEL. 1994. Adult cat flea (Siphonaptera: Pulicidae) excretion of host blood proteins in relation to larval nutrition. J. Med. Entomol. 31(2): 265-271.

STRENGER, A. 1973. Zur Ernahrungsbiologie der Larve von *Ctenocephalides felis felis*. B. Zool. Jb. Syst. 100: 64-80.

WADE, S. E., AND J. R. GEORGI. 1988. Survival and reproduction of artificially fed cat fleas, *Ctenocephalides felis* Bouche' (Siphonaptera: Pulicidae). J. Med. Entomol. 25(3): 186-190.

TRAPPING OF *SCYPHOPHORUS ACUPUNCTATUS* (COLEOPTERA:CURCULIONIDAE) WITH TWO NATURAL BAITS IN A FIELD OF *POLIANTHES TUBEROSA* (LILIALES:AGAVACEAE) IN THE STATE OF MORELOS, MÉXICO

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The plant locally called "tuberose", *Polianthes tuberosa* L. (Liliales: Agavaceae) is endemic to México. It is used to make flower ornaments and to extract volatile compounds for perfume manufacturing (Conzatti 1981; Watson & Dallwitz 1999). The black weevil *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) is a pest of *P. tuberose*. The highest percentage of plants damaged by this weevil, observed in Coatlán del Río, was reported as 69% (Camino et al. 2002a,b). It also attacks the *Agave salmiana* Otto ex. Salm-Dyck "pulque" and *A. fourcroydes* Lemaire "henequén" (MacGregor & Gutiérrez 1983; Morón & Terrón 1988). Ramírez-Choza (1993) found it in all regions where agave is cultivated. It is the main pest of sisal, causing damage of up to 50% of this crop. It has caused damage in "tequila" agave (*A. tequilana* Wever var. Blue), accounting for 10% loss of crops (Valenzuela 1994; Solís et al. 1999). Solís et al. (2001) reported up to 24.5% damage by *S. acupunctatus* in the *A. tequilana* heads. Recently in Morelos state "tequila" agave culture was introduced and Cabrera & Orozco (2002) reported that Counter (terbufos) controlled this insect in tuberose culture. Camino et al. (2000a, b) reported the use of 20 different bait types consisting of fruits and plant residues in two trap types (different from the ones used in the present work), where fermented agave, ripe pineapple, banana, and guava apple captured the highest number of adult *S. acupunctatus*; no natural baits were reported previously as attractive for this insect species.

This study was conducted to assess the response of *S. acupunctatus* towards two natural baits (Camino et al. 2002a), and the effectiveness of two trap types (a commercial one plus a funnel-type homemade one) for capturing adult weevils.

Fieldwork was conducted from August to October, 2001, in Morelos, México (18°53'N-99°11'W) at an altitude of 1350 m. A 1.2-ha parcel planted with offshoots of *P. tuberosa* was used as an experimental field and was divided into 18 plots (28 × 35m), with one trap placed in the center of each plot. A two-factor design (two trap types and two bait types) including six treatments and three replicates was used. The traps evaluated were the Victor (V) trap made of transparent plastic measuring 9 × 16 × 7 cm (depth:height:width) with a black cap having four 1 cm-diameter orifices at its base and with a yellow umbrella-shaped cap measuring 7 cm in diameter (Fig. 1a) and a yellow

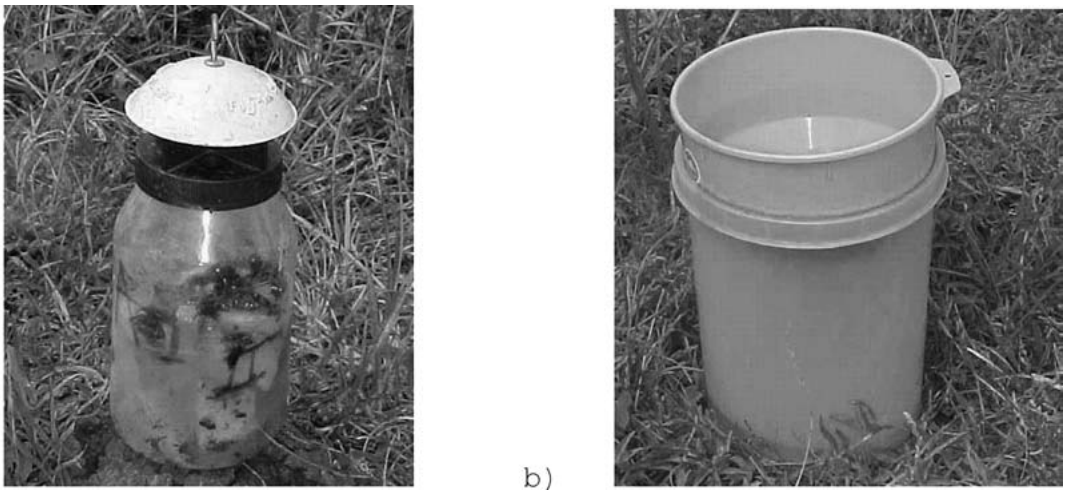

Fig. 1. Traps used for capturing the tuberoses black weevil *S. acupunctatus*: a) Victor trap, and b) Funnel trap.

funnel (F) trap consisting of a cylindrical 20 × 32 × 20-cm (depth:height:width) plastic container, with a plastic funnel measuring 25 × 27 × 22 cm (depth:height:width) (Fig. 1b). The bait types were ripe chopped pineapple (*Ananas comosus*) and fermented maguey (*A. salmiana*); additionally, water was used as control. The traps were randomly distributed. Baited traps were rotated at one-week intervals to avoid any position-related bias, for a total period of three months.

The nine V and F traps were baited as follows: three, with 300g of ripe, chopped pineapple plus 250 ml of water, three with 500 ml of fermented maguey, and three traps contained 250 ml of water only. Traps were rotated, checked every 8 days at 0900 h to collect adult weevils, and baits were replaced at each sampling interval. There were 10 sampling events. Captured weevils were placed in separate plastic containers and taken to the laboratory for counting.

The numbers of weevils captured were compared during the 3- month test by a two-way ANOVA. Means were separated by a Student Newman-Keuls test. Data were analyzed with the computer software SigmaStat for windows, version 2.03 (SigmaStat 1995).

Trap V captured significantly more weevils (a total of 1726 specimens) than trap F ($F = 7.620$, $df = 1,114$, $P = 0.007$) (Fig. 2) In August, the V trap captured 277 organisms, and the number increased to 385 in September, with maximum capture of 2232 in October. The V-trap design includes 4 orifices in the cap, which may lead to better release of volatiles. Weevils captured by the F trap were always lower than those for the V trap; however, the trend in counts over the course of the investigation were similar to those observed for V traps, namely less than 50 specimens captured in August and September followed by an increase in early October, with a peak totaling 2894 insects captured with both bait types, then decreasing and remaining at low numbers until the end of the experiment. This may be because of trap design, which only presents one opening for the volatilization of fermentation products. No captures were recorded in the control treatments throughout the experiment.

The fermented maguey was the most attractive bait for *S. acupunctatus* in August, but pineapple accounted for the highest counts in September-October. No statistical differences were observed between baits ($F = 0.106$, $df = 2,114$, $P > 0.05$) (Fig. 3). This might be explained by the fact that some of the fermentation products are identical or similar in both bait types; according to Figueroa et al. (2001) the major component in both pineapple and fermented maguey is ethanol, with minor components including acetaldehyde, acetic acid, and ethyl acetate. A difference between sampling events was detected ($P < 0.001$), with sampling 7 being the most significant one (748 insects). Data obtained for the sugar cane weevil *M. hemipterus sericeus* (Giblin-Davis et al. 1994a) and palm weevils *R. palmarum* and *R. cruentatus* (Camino et al. 1992; Oehlschlager et al. 1993; Giblin-Davis et al. 1994b; Oehlschlager et al. 1995) suggest that fragrances and fermentation products (ethyl acetate, ethyl lactate, ethyl isobutyrate, ethanol, butanol, acetic acid, hexanoic acid, and lactic acid) derived from a variety of plants or fruits are attractive for these insects.

The statistical comparison of traps and baits revealed no significant differences; however, substantial capture may contribute to decreasing the size of populations feeding on and damaging tuberose crops.

The Victor type was the most effective trap for capturing *S. acupunctatus*, suggesting that this

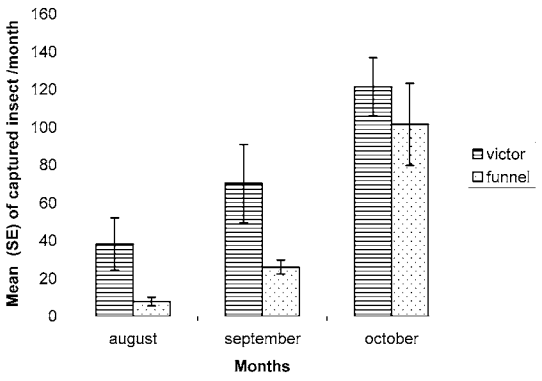

Fig. 2. Capture of *S. acupunctatus* adults in the field with two natural baited traps in Morelos Mexico ($F = 7.620$, $df = 1,114$, $P > 0.007$). Bars show SE.

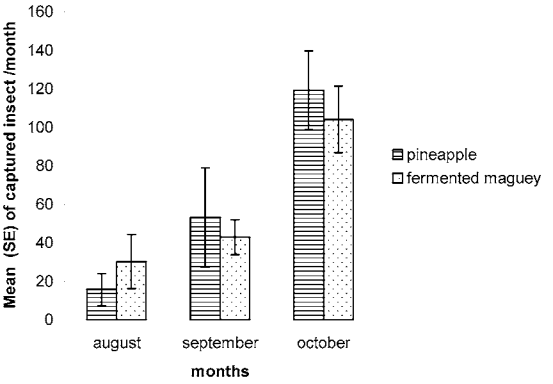

Fig. 3. Capture of *S. acupunctatus* adults in the field using two natural baits in Morelos Mexico. There is not statistical difference observed between fermented maguey and pineapple ($F = 0.606$, $df = 2,114$, $P > 0.05$). Bars show SE.

trap design allows a better volatile dissemination. Hence, this trap type can be recommended to monitor weevil populations, or as an aid to reduce the population size of this pest as part of an Integrated Pest Management program.

The results presented in this research work are part of the project CGPI-IPN 200091. The authors thank the Fundación Produce Morelos A.C. economic support received to carry out this investigation.

SUMMARY

Tests were conducted to assess the attraction of *Scyphophorus acupunctatus* to two natural baits (fermented maguey and pineapple) in two trap designs (Victor and funnel) in 2001 in fields planted with offshoots of *Polianthes tuberosa* in Emiliano Zapata, Morelos, México. There was a statistically significant difference between traps, with the Victor-type trap giving the largest catches.

REFERENCES CITED

CABRERA, R. J., AND M. R. OROZCO. 2002. Diagnóstico de ornamentales en el Estado de Morelos. INIFAP, CE Zacatepec. Zacatepec, Morelos. Publicación especial 31 p.

CAMINO, L. M., A. JIMÉNEZ, AND F. CASTREJÓN. 1992. Pruebas de atrayentes químicos para la captura de *Rhychophorus palmarum* (L.). Revista Latinoamericana de Química 23 (1): 11-13.

CAMINO, L. M., Y. RÍOS, R. FIGUEROA, M. GUTIÉRREZ, MA. C. HERNÁNDEZ, J. MARTÍNEZ, L. ALDANA, AND MA. E. VALDÉS. 2000a. "Manejo integrado de plagas del nardo *Polianthes tuberosa* (Liliflorae: Amarillidae) en el estado de Morelos." Informe Técnico. Fundación Produce Morelos A. C.

CAMINO, L. M., M. GUTIÉRREZ, R. FIGUEROA, L. ALDANA, MA. E. VALDÉS, MA. C. HERNÁNDEZ, AND R. URIBE. 2000b. Avances para la propuesta del programa MIP del picudo negro del nardo y agave en el estado de Morelos. En: Memorias VII Encuentro de Entomólogos del IPN CIIDIR-Oaxaca, Oaxaca, México, 31 October—1 November 2000: 25 p.

CAMINO, L. M., L. ALDANA, MA. E. VALDÉS, R. FIGUEROA, MA. C. HERNÁNDEZ, M. GUTIÉRREZ, AND J. MARTÍNEZ. 2002a. Bases para un programa MIP en el cultivo de nardo (*Polianthes tuberosa*) y agave (*Agave tequilana* var. azul) en el estado de Morelos, México. En: Resúmenes VIII Congreso Latinoamericano y del Caribe de Manejo Integrado de Plagas. Panamá, Panamá, 22-24 November 2002: 31 p.

CAMINO, L. M., V. CASTREJÓN, R. FIGUEROA, L. ALDANA, AND MA. E. VALDÉS. 2002b. *Scyphophorus acupunctatus* (Coleoptera: Curculionidae) attacking *Polianthes tuberosa* (Liliales: Agavaceae) in Morelos, México. Florida Entomol. 85 (2): 392-393.

CONZATTI, C. 1981. Flora taxonómica Mexicana II. Ceneti; Guadalajara, México. (see p. 87-88).

FIGUEROA, B. R., M. CAMINO, J. MARTÍNEZ, E. LAGUNAS, T. HERNÁNDEZ, Y. RÍOS, L. ALDANA, AND MA. E. VALDÉS E. 2001. Evaluation of natural products in traps for the capture of the *Scyphophorus acupunctatus* (Coleoptera: Curculionidae) in tuberose *Polianthes tuberosa* (Liliiflorae: Amaryllidaceae) from Morelos, México. 42nd Annual Meeting of the American Society of Pharmacognosy. Oaxaca, México, 14-18 July 2001: 243 p.

GIBLIN-DAVIS, R. M., J. PEÑA, AND R. DUNCAN. 1994a. Lethal pitfall trap for evaluation of semiochemical mediated attraction of *Metamasius hemipterus sericeus* (Coleoptera: Curculionidae). Florida Entomol.77 (2): 247-255.

GIBLIN-DAVIS, R. M., T. WEISSLING, A. OEHLSCHLAGER, AND L. GONZALEZ. 1994b. Field response of *Rhynchophorus cruentatus* (Coleoptera: Curculionidae) to its aggregations pheromone and fermenting plant volatiles. Florida Entomol.77 (1): 164-177.

MACGREGOR, R., AND Y. O. GUTIÉRREZ. 1983. Guía de insectos nocivos para la agricultura en México. Ed. Alhambra Mexicana. 166 p.

MORÓN, M. A., AND Y. R. TERRÓN. 1988. Entomología practica. Instituto de Ecología. Jalapa, México. (see p. 288-289).

OEHLSCHLAGER, A. C., C. CHINCHILLA-LÓPEZ, C. GONZÁLEZ, L. JIRÓN-PORRAS, L MEXSÓN-VARGAS, AND R. MORGAN. 1993. Development of a pheromone-based trapping system for *Rhynchophorus palmarum* (Coleoptera: Curculionidae). J. Econ.Entomol.86 (5): 1381-1392.

OEHLSCHLAGER, A. C., R. MCDONALD, C. CHINCHILLA-LÓPEZ, AND S. PATSCHKE. 1995. Influence of a pheromone-based mass-trapping system on the distribution of *Rhynchophorus palmarum* (Coleoptera: Curculionidae) in oil palm. Environ. Entomol. 24 (5): 1005-1012.

RAMÍREZ-CHOZA, J. L. 1993. Max del henequén *Scyphophorus interstitialis* bioecología y control. Serie libro técnico. Centro de Investigación Regional del Sureste. INIFAP-SARH. Mérida, Yucatán, México.

SIGMASTAT. 1995. Versión 2.03. Access, Soften Inc., San Rafael, CA.

SOLÍS, A. J., H. GONZÁLEZ, AND F. FLORES. 1999. Insectos asociados con *Agave tequilana* var. azul en cinco localidades de Jalisco, México. En: Memorias XXXIV Congreso Nacional de Entomología, Aguascalientes, Aguascalientes. 23-26 May 1999: 455-457.

SOLÍS, A. J. 2001. El picudo del agave tequilero *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) en Jalisco, México. Tesis Doctoral. Colegio de Postgraduados. Instituto de Fitosanidad. Montecillo, Texcoco, Edo. de México. 93 p.

VALENZUELA, Z. A. G. 1994. El agave tequilero. Ed. Litteris. (See p. 21-137).

WATSON, L., AND M. DALLWITZ. 1999. The families of flowering plants: descriptions, illustrations, identification and information retrieval. (On line) Available: *http://biodiversity.uno.edu/delta/* (4 November 2000).

PEDARIDIUM MAYA (COLEOPTERA: SCARABAEIDAE): FIRST RECORD IN YUCATAN, MEXICO

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Pedaridium maya Vaz de Melo, Halffter & Halffter (2004) was described recently from Mexico (Quintana Roo, Chiapas, and Campeche) and Guatemala and is known nowhere else. It is isolated geographically and morphologically from other species of the genus. During a year of monthly systematic samplings (August 2001 to July 2002) in Tigre Grande, Tzucacab, Yucatan (19°42'36" N, 89°02'28"W), three permanent 'necro' traps and three temporary 'copro' traps (Morón & Terrón 1984) captured five adult specimens of *P. maya*. Collection data are: MEXICO: YUCATAN, Tzucacab, Tigre Grande, 20-VIII-17-IX-2001, NTP80, L.N. Peraza, col. (1f); MEXICO: YUCATAN, Tzucacab, Tigre Grande, 15-V-12-VI-2002, NTP80, L.N. Peraza, col. (2m, 1f); MEXICO: YUCATAN, Tzucacab, Tigre Grande, 10-12-VI-2002, copro trampa temporal, L.N. Peraza, col. (1f).

The specimens are deposited in the entomological collection of the Instituto de Ecología, A.C. (IEXA), Xalapa, Veracruz. They represent the first records for the State of Yucatan and were captured in May, June, and August in tropical medium subperennifolious forest at 70 m altitude, with annual precipitation between 1000 and 1200 mm. In Tigre Grande, *P. maya* was captured with 26 other species of Scarabaeidae of the genera *Canthon* Hoffmansegg (3 species), *Deltochilum* Eschscholtz (3), *Pseudocanthon* Bates (1), *Sisyphus* Latreille (1), *Eurysternus* Dalman (1 species), *Onthophagus* Latreille (6 species), *Copris* Müller (2 species), *Dichotomius* Hope (1 species), *Canthidium* Erichson (2 species), *Ateuchus* Weber (1 species), *Coprophanaeus* Olsoufieff (1 species), *Phanaeus* MacLeay (2 species), and *Uroxys* Westwood (2 species).

This communication is a contribution to the project "Systematics and ecology of phytophagous and saprophagous insects" of the Departamento de Entomología (902-08), Instituto de Ecología, A.C. (CONACYT).

SUMMARY

Pedaridum maya Vaz de Melo, Halffter & Halffter is recorded for the first time for the State of Yucatan with five adult specimens captured in Tigre Grande, Tzucacab, Yucatán, Mexico in monthly systematic sampling during one year (August 2001 to July 2002).

REFERENCES CITED

MORÓN, M. A., AND R. A. TERRÓN. 1984. Distribución altitudinal y estacional de los insectos necrófilos de la Sierra Norte de Hidalgo, México. Acta Zool. Mexicana (n.s.) 3: 1-47.

VAZ DE MELO, F. Z., G. HALFFTER, AND V. HALFFTER. 2004. A new species of *Pedaridium* Harold from Mexico and Guatemala (Coleoptera: Scarabaeidae: Scarabaeinae: Coprini: Ateuchina). Coleopterist's. Bull. 58: 247-252.

NOTES ON A NATIVE AND AN EXOTIC SCARAB COLLECTED IN GUERRERO, MEXICO (COLEOPTERA: SCARABAEIDAE)

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During collections carried out in Guerrero, Mexico in 2001, *Euoniticellus intermedius* (Reiche 1849), a scarab not previously known from Guerrero was captured. In addition, *Eurysternus magnus* Castelnau 1840 was collected in Acahuizotla on September 4, 2001 at an altitude of 650 m, a range extension for this native species.

Euoniticellus intermedius (Reiche 1849) was introduced into Texas in the 1970s. The first record from Mexico was from the Reservation of the Biosphere "La Michilía", in the state of Durano by Montes de Oca et al. (1994). Three years later, Montes de Oca & Halffter (1997) reported it from Baja California Sur, Guanajuato, Michoacán, Chihuahua, Tamaulipas, Hildago, and Veracruz. Deloya (2000) recorded it from the south of the state of Morelos. In monthly collections carried out in the Sierra Madre del Sur between 1,400-1,670 between January and June, 2001 at Ocotito (17°14'17" N, 99°30'43" W), Guerrero, a female specimen of *E. intermedius* was collected. It is the first record for the state of Guerrero. The specimen is labelled "MEXICO: Guerrero, Chilpancingo, Ocotito, 30-III-2001, altitude 680 m, bovine excrement, 12:30 pm, E. Ramírez, col. (1)". It is deposited in the entomological collection of the Instituto de Ecología, A.C. (IEXA). The locality is a *Pinus-Quercus* forest with annual precipitation of 1650 mm and annual half temperature of 24°C.

The native scarab *Eurysternus magnus* has a wide distribution between Panama and Mexico in montane rain forest between the 900 and 2,200 m (Howden & Young 1981; Jessop 1985; Morón 1994). In Mexico, it has been reported from Chiapas, Guerrero Hidalgo, Oaxaca, Querétaro, Tamaulipas, and Veracruz. In the Sierra Madre del Sur, *E. magnus* had been recorded from Juquila, Oaxaca to 1,550 m (Bates 1888) and from the Sierra del Alquitrán, Guerrero, in a *Quercus-Pinus* forest between 1,400 and 1,670 m (Delgado 1997).

Delgado Castillo (1989) carried out a study between 1985 and 1987 on the Scarabaeoidea fauna of Acahuizotla, between 650-850 m altitude. He mentions *Pseudocanthon* Bates, *Deltochilum* Eschscholtz, *Canthon* Hoffmansegg, *Copropha-naeus* Olsoufieff, *Phanaeus* MacLeay, *Copris* Müller, *Dichotomius* Hope, *Canthidium* Erichson, *Ateuchus* Weber, *Scatimus* Erichson, *Uroxys* Westwood, and *Onthophagus* Latreille. In that study, intensive sampling with pitfall traps baited with carrion, bovine dung, and equine dung failed to reveal the presence of *Eurysternus*.

A specimen of *Eurysternus* now deposited in the entomological collection of the Instituto de Ecología, A.C. (IEXA), is labelled MEXICO: Guerrero, Achauizotla, 650 m msm, 4-IX-2001, Juarez Pineda, A.J. col. (1)." This specimen was collected at substantially lower altitude (650m) than specimens reported by previous collectors, who collected at 1400-1670 m.

This communication is a contribution to the project "Systematic and ecology of insects phytophagous and saprophagous" of the Departamento Entomología (912-044), Instituto de Ecología, A.C.

SUMMARY

Euoniticellus intermedius (Reiche) is reported for the first time from the State of Guerrero, Mexico, with a female specimen captured at Ocotito on 30 March 2001. The altitudinal range of *Eurysternus magnus* Castelnau in the Sierra Madre del Sur (1,400-1,670 m), is greatly extended with a male specimen captured at 650 m at Acahuizotla, Guerrero, on September 4, 2001.

REFERENCES CITED

BATES, H. W. 1886-1890. Pectinicornia & Lamellicornia. Biologia Centrali-Americana, Insecta Coleoptera II(2): 1-432.

DELGADO CASTILLO, L. L. 1989. Fauna de coleópteros lamelicornios de Acahuizotla, Guerrero, México. Tesis Licenciatura, Facultad de Ciencias, UNAM, México, 154 pp.

DELGADO, L. 1997. Distribución estatal de la diversidad y nuevos registros de Scarabaeidae (Coleoptera) mexicanos. Folia Entomológica Mexicana 99: 37-56.

DELOYA, C. 2000. Escarabajos exóticos (Coleoptera: Scarabaeidae) para la fauna de los estados de Morelos y Oaxaca, México. Folia Entomol. Mexicana 108:125-126.

HOWDEN, H. F., AND O. P. YOUNG. 1981. Panamian Scarabaeinae: Taxonomy, distribution and habitats (Coleostera, Scarabaeidae). Contrib. American Entomol. Inst. 18(1): 1-204.

JESSOP, L. 1985. An identification guide to Eurysternine dung beetles (Coleoptera, Scarabaeidae). J. Nat. Hist. 19: 1087-1111.

MONTES DE OCA, E., S. ANDUAGA, AND E. RIVERA. 1994. Presence of the exotic dung beetle *Euoniticellus intermedius* (Reiche) in northern Mexico. The Coleopterist's Bulletin 48(3): 244.

MONTES DE OCA, E., AND G. HALFFTER. 1997. Invasion of Mexico by dung beetles previously introduced into the United States. Stud. Neotrop. Fauna Environ. Vol. 33: 37-45.

MORON, M. A. 1994. Fauna de Coleoptera Lamellicornia en las montañas del Noreste de Hidalgo. Acta Zoológica Mexicana (n.s.) 63: 7-59.

IFKOVIC, E. 2004. The Life and Works of Writer Annie Trumbull Slosson - a Connecticut Local Colorist. Studies in American Literature Vol. 68. Edwin Mellen Press; Lewiston, NY. viii + 481 pp. ISBN 0-7734-6396-8. Hardback. $139.95

Female entomologists were a rarity in the nineteenth century. In his biography of Annie Trumbull Slosson, Edward Ifkovic describes the times and circumstances that led to the entomological pursuits of one of the greatest. Born in 1838 to a wealthy New England family that encouraged writing, women's education, and the study of science, she became a keen naturalist with the means to follow good weather and collect from New Hampshire to southern Florida, often accompanied by fellow botanists and entomologists. Her accomplishments as an "amateur" entomologist are astonishing, made more so by her entry into the field at the advanced age of 48. Despite continual donations of insect specimens to collectors and taxonomists, she amassed a collection of 35,000 specimens, which she donated to the American Museum of Natural History. She described several species, and over a hundred species were named for her. She was a founding member and financial supporter of the New York Entomological Society, and was instrumental in launching its journal. Her many publications contributed to insect taxonomy and systematics, life histories, faunistic surveys, and philosophy. Her extensive collection of entomological correspondence was catalogued. A year before her death in 1926, the Brooklyn Entomological Society elected her an Honorary Member.

Using a wealth of correspondence, personal journals, published works, and archival material, Ifkovic provides a richly detailed account of Slosson's life and accomplishments. He deftly resurrects the character of an eccentric woman who, at middle age, was poised on the brink of greatness as a local color writer but fell instead under the spell of entomology, severely curtailing her output of fiction. In the introductory chapters he describes the literary style called local color that thrived in the United States after the Civil War and lingered through the turn of the century. The term "local color" certainly applies to Slosson's work. The style centered on rural life and closeness to nature, capturing the rough dialects of village folk and details of the fauna and flora of the story setting. In stark contrast to the romantic sentimentalist style that it was supplanting, local color, especially from New England, often celebrated single older women whose lives revolved not around men but on their own strengths. Not surprisingly, the best of the local colorists were single women, including the widowed Slosson. And not unexpectedly, the style became popular during the second half of the nineteenth century, when women were allowed to pursue higher education and the suffrage movement was in full force.

Within the introductory chapters, Ifkovic describes the social, economic, literary and religious matrix of Hartford, Connecticut, during the 1800s, and the activities of the influential Trumbulls and their extended family. Together they form a backdrop for Slosson's development as a writer and scientist. Annie's parents were progressive Calvinists who advocated women's education and sent her to the Hartford Female Seminary, which had a strong curriculum in science, unusual in a girls' school at that time. She remained religious all her life, but promulgated in her fiction and spiritual essays a shift toward more liberal and sensible Calvinism, reflecting the currents of the day.

In subsequent chapters, Ifkovic analyzes Slosson's major works of fiction in chronological context. Many of her popular stories first appeared in 'The Atlantic Monthly' and 'Harper's Bazaar', and were later collected and published in book form. Some were written as novels. They ranged, for instance, from a loose conglomeration of essays and stories on a club of women who collect china, to the story of a woman who decides to forgo her place in heaven because her beloved animals are unfairly excluded from it. Ifkovic points out that the seemingly sentimental stories were satires and parables, reflecting mainstream changes in spirituality and Slosson's belief in the redeeming influence of woman's sensibility. After an evaluation of one of Slosson's later works, a story titled Dumb Foxglove, Ifkovic offers an eloquent summary that can serve for most of her local color writing: "Slosson shows that nature—this time the flower called Dumb Foxglove—is symbolic metaphor for human experience. The story exhibits what Slosson does best, meshing her naturalistic vision with the eccentricities of the isolated New England village, seen through a glass darkly illuminated by the peculiarities of religious zeal".

Slosson's interest in insects began around the time of her first successful fiction publications, and probably stemmed from her well-established passion for plant collecting and floral surveys. In the manner of the day, she called herself an "amateur botanizer", but was well versed in methods of identification and curation, and regularly corresponded with eminent botanists such as Asa Gray. She was actually an accomplished botanist who published floral surveys and species descriptions. Ifkovic suggests that Slosson was coaxed to study insects by her brother-in-law and companion, William C. Prime, a well-known writer, editor, trout fisherman, and lepidopterist. After the deaths of their spouses, the two established a routine of travel and collecting, summering in the White Mountains of New Hampshire, and winter-

ing in the rich collecting grounds of Florida, with interludes in New York and Hartford. Slosson's interest in insects was encouraged also by another close friend, the actor and entomologist Henry Edwards, who fueled her growing passion by naming a moth for her, a species she collected in Florida. She was hooked. "You know what an insidious, enthralling, captivating habit it is. . . What are drugs to bugs!" She established correspondence and friendships with a veritable Who's Who of early entomology, among them W. A. Ashmead, Philip P. Calvert, D. W. Coquillet, Edward P. van Duzee, Harrison G. Dyar, A. S. Packard, and Henry Skinner. Her passion for what she informally called "bugology" was so intense that she apparently carried a cyanide jar even to church, and admitted to using it to collect a small moth from the pew in front of her one Sunday while on her knees in prayer. This was recounted in a lighthearted essay published by Bradford Torrey, an ornithologist and another close friend. Ifkovic provides several humorous excerpts by and about Slosson that highlight her thrill with the eccentricity of being an entomologist, and a female one at that!

Slosson's literary contributions to entomology include philosophical essays on a variety of topics, such as the use of common versus scientific names, and the meshing of entomology and literature. Her descriptive articles were written in a more literary style, to, in her words, "relieve the heaviness of the masculine articles." The lively pieces, says Ifkovic, were "filled with engaging anecdote and wry observation", and were well received by her colleagues. He sums up her articles as ". . . always filled with human-instinct vignette, her fusion of the drama of literature with the circumstances of entomology".

Beside the introduction and eleven chapters of Slosson's biography, Ifkovic presents notes, an extensive bibliography of Slosson's publications and other sources, an index, and a selection of photographs and illustrations. Ronna Coffey Privett, an authority on British and American literature, ably wrote the preface. The book offers a thorough and engaging account of one of America's most colorful early entomologists, a woman who was once listed as a tourist attraction because of her habit of flailing a butterfly net in public places, whose wit and knowledge entertained many, and who contributed generously to the field and to the advancement of the careers of both young and established entomologists. Combined with a well-textured description of the times and places in which Slosson lived, wrote, and collected, the book has an offering for a wide range of readers, from biologists to sociologists and historians of literature.

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