OLIGIDIC DIETS FOR CULTURE OF *RHYNCHOPHORUS CRUENTATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Several artificial diets were evaluated as alternatives to decomposing pineapple [*Ananas comosus* (L.) Merrill] for culture of *Rhynchophorus cruentatus* (F.) larvae. The most suitable diet tested for larval growth and survival was a combination of canned pineapple, oats, sucrose, molasses, brewers yeast, Wesson's salts, vitamins, and preservatives. Diets that were not supplemented with brewers yeast provided poor larval growth and survival. Larvae cultured from artificial diets were placed in sugarcane (*Saccharum officinarum* L.) for pupation.

Key Words: Palm weevil, palmetto weevil, laboratory rearing, artificial diets.

RESUMEN

Varias dietas artificiales fueron evaluadas como alternativas a la piña descompuesta [*Ananas comosus* (L.) Merrill] para el cultivo de larvas de *Rhynchophorus cruentatus* (F.). La mejor dieta probada para el crecimiento y la supervivencia larval fué una combiación de piña enlatada, cebada, azúcar, melaza, levadura de cerveza, sales de Weson, vitaminas y preservativos. Las dietas sin levadura de cerveza produjeron poco crecimiento larval y sobrevivencia. Las larvas criadas en dietas artificiales fueron colocadas en caña de azúcar (*Saccharum officinarum* L.) para su pupación.

Rhynchophorus cruentatus (F.) is the only species of palm weevil in the continental United States (Wattanapongsiri 1966). Unlike several of its congeners, *R. cruentatus* is not considered a major pest of palms. However, this species will attack transplanted or otherwise stressed ornamental palms (Giblin-Davis & Howard 1988, 1989). In Florida, *R. cruentatus* is sympatric with the native cabbage palmetto, *Sabal palmetto* (Walter) Loddiges ex Schultes (Woodruff 1967), a palm often used as mature specimens in landscaping due to its low cost, natural abundance, and high transplanting survivorship.

Semiochemicals emanating from wounded or dying palms (Chittenden 1902, Wattanapongsiri 1966, Weissling et al. 1992, Giblin-Davis et al. 1994) and conspecific weevils (Weissling et al. 1993, 1994) are attractive to *R. cruentatus* adults. Females lay eggs in the leaf bases or directly into the wounds of dying host palms. The larvae molt several times and are voracious consumers of tissue within the bud and stem. Last-instar larvae migrate to the periphery of the host, prepare a cocoon from the fi-

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ber, and enter a pupal stage. Following eclosion, adults emerge from the tree. The life cycle of this weevil usually is completed in less than 84 d (see Giblin-Davis & Howard 1989 for detailed description).

Research on the biology of *R. cruentatus*, as well as its vector potential for the red ring nematode [*Bursaphelenchus cocophilus* (Cobb)], requires the collection of adults in the field, an expensive and time consuming endeavor. Thus, a laboratory rearing method for *R. cruentatus* was needed. Three other species of palm weevils have been reported to be cultured in the laboratory. *Rhynchophorus ferrugineus* (Olivier) (Rananavare et al. 1975) and *R. palmarum* (L.) (Wilson 1963) can be cultured using cut petiole or stem tissue of coconut palms, and *R. cruentatus* has been reared on buds of an alternate host, *Serrenoa repens* (Bartram) Small (Berger 1907). However, collection of palm tissue for culture of weevils is also expensive. Rahalkar et al. (1972) reported that sugarcane is a good substitute for coconut stem for rearing *R. ferrugineus*. This method was improved by incorporating sugarcane in nutrient agar for young larvae and whole sugarcane stem pieces for older larvae (Rananavare et al. 1975). Culture of *R. ferrugineus* was further improved by development of an artificial diet (Rahalkar et al. 1978, 1985) containing sugarcane bagasse (fiber), coconut cake, yeast, sucrose, minerals, vitamins, and preservatives. Using a combination of corn flour, sugarcane fiber, oats, sugar, coconut oil, and propionic acid for a larval medium and sugarcane for pupation, Sánchez et al. (1993) successfully cultured *R. palmarum*. Giblin-Davis et al. (1989) determined that *R. cruentatus* and *R. palmarum* could be cultured on decomposing pineapple [*Ananas comosus* Merrill] syncarp for young larvae and sugarcane stem for mature larvae. However, variation in the quality and availability of pineapple, and the inconvenience of working with decomposing material, prompted the development and evaluation of several agar-based diets for laboratory-culture of *R. cruentatus*.

Materials and Methods

Insects

R. cruentatus adults were harvested as cocoons from infested *S. palmetto*, placed individually in covered 100-ml plastic cups with moistened tissue paper (Giblin-Davis et al. 1989) and stored at 29 °C until adult emergence. Females and males were placed as individual pairs in 500-ml covered containers with moistened tissue paper and a slice of apple (*Pyrus malus* L.). The apple slices were replaced at 1-3 day intervals, carefully dissected, and the eggs removed (Weissling & Giblin-Davis 1994). Eggs were transferred to petri dishes (15 × 100 mm) lined with moistened filter paper, sealed with parafilm, and stored at 29 °C until neonate larvae emerged.

Diet Preparation

All diets included 50 g bacto-agar (Difco Laboratories, Detroit, MI), 12.5 crushed vitamin tablets (Centrum; Lederle Laboratories Div., Pearl River, NY), and 1892 ml water. Each vitamin tablet weighed 1.4 g and contained: vitamins A (5000 I.U.), E (30 I.U.), C (60 mg), B_1 (1.5 mg), B_2 (1.7 mg), B_6 (2 mg), B_{12} (6 µg), D (400 I.U.), and K_1 (25 µg), folic acid (400 µg), niacinamide (20 mg), biotin (30 µg), pantothenic acid (10 mg), calcium (162 mg), phosphorus (125 mg), iodine (150 µg), iron (18 mg), magnesium (100 mg), copper (2 mg), zinc (15 mg), manganese (2.5 mg), potassium (40 mg), chloride (36.3 mg), chromium (25 µg), molybdenum (25 µg), selenium (25 µg), nickel (5 µg), tin (10 µg), silicon (10 µg), and vanadium (10 µg). In addition, all diets contained the pre-

servatives: m-para-hydroxybenzoate [14% solution in 95% ethyl alcohol (25 ml)], sorbic acid [12.5% solution in 95% ethyl alcohol (37.5 ml) in preliminary tests or 6.28 g sorbic acid potassium salt in refined diets], and 4M potassium hydroxide solution (7.5 ml) (Rahalkar et al. 1985). All other ingredients and amounts are listed in Table 1. All materials except the crushed vitamin capsules and a 500-ml aliquot of water were blended for approximately 2 min and poured into a 5.7-liter stainless-steel bowl. The blender was rinsed with the remaining water and poured into the bowl. The mixture was then autoclaved for 20 min at 120 °C. As the diet cooled, the crushed vitamin tablets were added and the media was stirred. Diets were poured into diet cups while still warm. When diets cooled, a small hole was made in the diet surface to facilitate feeding and larvae were transferred one per diet cup with a fine camel hair brush. Diet cups were covered with a lid vented by several small holes. After 24 h, diets were checked and dead larvae were replaced. All tests were conducted in an environmental chamber at 29 °C with a photoperiod of 13:11 (L:D).

Preliminary Evaluation of Diets

Ten to twenty replicates of seventeen different diets (Table 1) were evaluated in preliminary tests for larval and adult biomass gain and survival. We also included the diet for culture of *R. ferrugineus* (Rahalkar et al. 1985). Experimental diets consisted of 100 g of diet in 100-ml cups. Preliminary observations indicated the need for fresh diet if larvae were left in culture for more than three weeks (unpublished data). Thus, larvae fed diets 1-17 and the published diet were removed from cups after three weeks and transferred to 100 g of fresh diet. During this transfer, larvae were rinsed with water, patted dry with paper toweling, and weighed. After an additional two weeks, larvae were removed, cleaned, and reweighed. To determine if larvae could be cultured for more than three weeks without a change in diet, we included diet 18 which was 200 g of diet 3. However, 100-ml cups were too small for this amount of diet so the media was placed in 500-ml cups. Larvae on diet 18 remained undisturbed for five weeks, at which time they were cleaned and weighed. After the final weighing, larvae from all diets were individually placed into holes (0.95 cm diam) drilled 10 cm into one end of 23-25 cm lengths of sugarcane (Giblin-Davis et al. 1989). Stems were wrapped in window screen secured at each end by elastic bands, and placed in an environmental chamber at 29 °C. After three weeks, stems were split open and cocoons, if present, were removed and placed in 100-ml vented cups with moistened tissue paper and stored at 29 °C until adult emergence. If larvae were still present, the cane was carefully re-wrapped, placed back in the environmental chamber, and checked one week later.

Culture Technique Modifications

The biomass and survival of larvae reared for three weeks on diets 2, 3, 12, 13, and 15 was good in preliminary tests (Table 2). Therefore, larvae were tested again on these diets when reared in 100-ml cups (100 g of diet) and then transferred to sugarcane after three weeks. This was done to determine if healthy weevils were produced under this time and resource saving regimen. Based on acceptable growth of larvae cultured on diet 18 in 500-ml cups (Table 2), we also compared larval growth on the diets described above when 200 g of media was placed in 500-ml cups and the larvae were allowed to feed undisturbed for five weeks before transfer to sugarcane. Based on poor biomass gain after three weeks in culture, but high final biomass after five weeks in preliminary tests (Table 2), larvae fed diets 14 and 17 were evaluated

TABLE 1. INGREDIENTS USED TO PREPARE DIETS FOR CULTURE OF *R. CRUENTATUS*[1].

Ingredient	Diet No. and g of Each Ingredient																		
	Pub.[2]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18[3]
Canned palm hearts[4]	—	—	—	—	—	—	—	—	—	522.5	—	—	—	317.9	—	—	—	—	—
S. palmetto tissue	—	522.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sugarcane bagasse	132.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Coconut cake	150.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Fresh potatoes	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	317.9	—
Canned pineapple[5]	—	—	162.5	161.5	143.7	142.7	280.4	—	142.7	—	485.0	522.5	161.5	—	317.9	—	472.5	—	161.5
Brewers yeast[6]	50.0	—	50.0	50.0	50.0	50.0	50.0	50.0	50.0	—	—	—	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Sucrose	190.0	—	125.0	125.0	125.0	125.0	125.0	125.0	125.0	—	—	—	125.0	125.0	125.0	125.0	—	125.0	125.0
Molasses[7]	—	—	65.0	65.0	65.0	65.0	65.0	65.0	65.0	—	—	—	65.0	65.0	65.0	65.0	—	65.0	65.0
Soybean oil	—	—	—	—	37.5	37.5	37.5	37.5	37.5	—	37.5	—	—	—	—	—	—	—	—
Cholesterol	—	—	—	2.0	—	2.0	2.0	2.0	—	—	—	—	—	2.0	2.0	2.0		2.0	2.0
Egg yolk	—	—	—	—	—	—	—	—	10.0	—	—	—	10.0	—	—	—	—	—	—
Oats[8]	—	—	157.5	156.5	138.7	137.7	—	280.4	137.7	—	—	—	156.5	—	—	317.9	—	—	156.5
Wesson's salts[9]	5.0	—	5.0	5.0	5.0	5.0	5.0	5.0	5.0	—	—	—	5.0	5.0	5.0	5.0	—	5.0	5.0

[1]All diets contained 50 g bacto agar, 12.5 crushed vitamin capsules, 25 ml of a 14% m-para-hydroxy benzoate stock solution, 37.5 ml of a 12.5% sorbic acid stock solution, 7.5 ml of a 4 M potassium hydroxide stock solution, and 1892 ml water (100 g diet in 100 ml cup).
[2]Artificial diet published by Rahalkar et al. (1978, 1985) for culture of *R. ferrugineus*.
[3]Diet 3; 200 g in 500 ml cup, without three week subculture.
[4]Source: Costa Rica.
[5]Pineapple chunks in unsweetened pineapple juice; Dole Packaged Foods Corp.
[6]Debittered NF (Entomologically tested); Bio-Serv, Frenchtown, NJ.
[7]Grandma's Molasses "unsulphured", Mott's USA, Stamford, CT.
[8]Quick Oats; The Quaker Oats Co., Chicago, IL.
[9]Salt mixture, Wesson modification; United States Biochemical Corp., Cleveland, OH.

TABLE 2. BIOMASS AND SURVIVAL[1] OF *R. CRUENTATUS* LARVAE AND ADULTS FED ARTIFICIAL DIETS (PRELIMINARY TESTS[2]).

		Larvae			Adults		
Diet	n	Biomass (g) 3 Weeks	Biomass (g) 5 Weeks	Survival %	Biomass (g)	Emergence (%)	Total Days in Sugarcane
Pub[3]	20	1.14 ± 0.17 de	2.87 ± 0.23 ab	70.0 ± 10.5 abc	0.89 ± 0.09 bc	50.0 ± 11.5 ab	50.6 ± 1.9
1	10	0.02 ± 0.00 e	0.02 ± 0.00 c	10.0 ± 0.0 cd	—	0.0 ± 0.0 b	—
2	20	2.82 ± 0.18 abcd	3.52 ± 0.19 ab	50.0 ± 11.5 abcd	1.07 ± 0.15 bc	30.0 ± 10.5 ab	48.5 ± 1.1
3	20	2.36 ± 0.18 abcd	3.25 ± 0.26 ab	65.0 ± 10.9 abc	1.06 ± 0.04 bc	45.0 ± 11.4 ab	50.0 ± 1.2
4	10	1.67 ± 0.14 bcde	2.90 ± 0.21 ab	30.0 ± 15.3 bcd	0.90 ± 0.10 bc	30.0 ± 15.3 ab	48.7 ± 2.0
5	10	1.89 ± 0.29 abcd	—	0.0 ± 0.0 d	—	0.0 ± 0.0 b	—
6	10	1.15 ± 0.00 de	2.90 ± 0.00 ab	10.0 ± 10.0 cd	0.70 ± 0.00 bc	10.0 ± 10.0 ab	51.0 ± 0.0
7	10	1.65 ± 0.34 bcde	—	0.0 ± 0.0 d	—	0.0 ± 0.0 b	—
8	10	2.33 ± 0.00 abcd	2.67 ± 0.00 ab	10.0 ± 10.0 cd	0.80 ± 0.00 bc	10.0 ± 10.0 ab	52.0 ± 0.0
9	20	1.38 ± 0.18 de	2.45 ± 0.18 b	70.0 ± 10.5 abc	0.83 ± 0.03 bc	55.0 ± 11.4 ab	50.2 ± 1.4
10	10	1.47 ± 0.43 cde	2.68 ± 0.02 ab	30.0 ± 15.3 bcd	0.80 ± 0.15 bc	30.0 ± 15.3 ab	53.7 ± 1.2
11	20	0.05 ± 0.01 e	0.10 ± 0.02 c	55.0 ± 11.4 abcd	0.50 ± 0.00 c	5.0 ± 5.0 b	65.0 ± 0.0
12	11	3.15 ± 0.30 ab	3.28 ± 0.36 ab	54.5 ± 15.7 abcd	1.07 ± 0.07 bc	27.3 ± 14.1 ab	48.7 ± 3.3
13	10	3.11 ± 0.33 abc	4.04 ± 0.16 ab	70.0 ± 15.3 abc	1.08 ± 0.03 bc	40.0 ± 16.3 ab	49.3 ± 2.7
14	10	1.24 ± 0.21 de	3.13 ± 0.19 ab	100.0 ± 0.0 a	1.04 ± 0.07 bc	70.0 ± 15.3 a	52.0 ± 1.0
15	10	3.40 ± 0.21 a	4.29 ± 0.34 a	40.0 ± 16.3 abcd	1.60 ± 0.20 a	20.0 ± 13.3 ab	52.5 ± 0.5
16	10	1.64 ± 0.30 bcde	3.28 ± 0.17 ab	80.0 ± 13.3 ab	1.18 ± 0.06 b	50.0 ± 16.7 ab	54.0 ± 0.7
17	10	1.15 ± 0.23 de	3.05 ± 0.31 ab	80.0 ± 13.3 ab	1.01 ± 0.05 bc	60.0 ± 16.3 ab	52.6 ± 0.7
18	10	—	3.99 ± 0.20 ab	80.0 ± 13.3 ab	1.12 ± 0.05 b	60.0 ± 16.3 ab	49.2 ± 2.0

[1]Means ± SEM.
[2]Means within a column followed by the same letter are not significantly different (P > 0.05; SNK).
[3]Artificial diet published by Rahalkar et al. (1978, 1985) for culture of *R. ferrugineus*.

further in 500-ml cups with 200 g diet. The biomass of these larvae was determined at five weeks before immediate transfer to sugarcane. Each diet and cup size combination was replicated 20 to 40 times.

Statistics

Larval and adult survival were based on the initial number of test insects. All data except percentage values were square root (X + 0.001) transformed. Percentage data were subjected to angular [arcsin (square root + 0.001)] transformation. Transformed data were subjected to analysis of variance using the Statistical Analysis System's general linear models procedure (SAS Institute 1985) for overall comparison of diets. Means were separated by Student Newman-Keuls' (SNK) test where significant ($P < 0.05$) effects occurred. Diets evaluated in 100-ml cups were compared with diets in 500-ml cups using orthogonal contrasts (SAS Institute 1985).

RESULTS

Preliminary Evaluation of Diets

After five weeks in culture the average biomass of larvae and adults, as well as larval survival and percentage adult emergence, varied greatly (Table 2). The greatest mean larval biomass was observed for diet 15 both at three (3.40 g) and five (4.29 g) weeks but survival was relatively low (40%) (Table 2). The greatest larval survival (100%) occurred with larvae fed diet 14; mean biomass at five weeks (3.13 g) was not significantly different from that of diet 15 (Table 2). There was little gain in biomass of larvae fed diets 1 and 11, however, survival on diet 11 was relatively high (55%), whereas survival on diet 1 was low (10%) (Table 2). Adult emergence was greatest for diet 14 (70%) while no adults emerged from larvae fed diets 1, 5, and 7 (Table 2). Total time from placement of larvae in sugarcane to adult emergence varied from 48.5 days (diet 2) to 65 days (diet 11) (Table 2).

Culture Technique Modifications

Overall, the greatest larval biomass was obtained from diet 2 in 500-ml (large) cups (3.5 g) while larvae fed diet 17 had the least biomass (1.39 g) (Table 3). The greatest larval survival was on diet 14 (92.5%) (Table 3). Adults reared as larvae on diets 2 and 3 (large cups) had the greatest biomass (0.94 and 0.92 g, respectively) while the greatest adult emergence was from larvae fed diet 12, small cups (55%) (Table 3). Total time from placement of larvae in sugarcane to adult emergence ranged from 33.8 days (diet 12, 500-ml cup) to 46.6 days (diet 17) (Table 3). Orthogonal contrasts indicated that overall larval and adult mass was greater for individuals cultured in 500-ml versus 100-ml cups ($P < 0.01$). However, adult emergence was greater for larvae cultured in 100-ml cups ($P < 0.03$).

DISCUSSION

Using the diet described by Rahalkar et al. (1978, 1985), and several variations of this diet using readily available materials, we were able to achieve growth and development of *R. cruentatus* larvae. Sugarcane stem was, however, necessary for completion of the life cycle. Of diets tested in the preliminary experiments, seven were

TABLE 3. BIOMASS AND SURVIVAL[1] OF *R. CRUENTATUS* LARVAE AND ADULTS CULTURED ON ARTIFICIAL DIETS[2].

Diet	Diet Cup Size[3]	*n*	Larval Biomass (g)	Larval Survival (%)	Adult Biomass (g)	Adult Emergence (%)	Total Days in Sugarcane	Males: Females
2	small	40	2.53 ± 0.10 c	87.5 ± 5.3 a	0.81 ± 0.04 abc	50.0 ± 8.0 a	43.7 ± 0.4	0.73
3	small	40	2.27 ± 0.12 cd	77.5 ± 6.7 ab	0.76 ± 0.04 abc	47.5 ± 8.0 a	44.8 ± 0.8	1.71
12	small	40	2.44 ± 0.11 c	70.0 ± 7.3 ab	0.79 ± 0.04 abc	55.0 ± 8.0 a	44.2 ± 0.9	1.44
13	small	40	2.10 ± 0.15 cd	60.0 ± 7.8 abc	0.70 ± 0.01 bc	45.0 ± 8.0 a	43.4 ± 0.6	0.80
15	small	40	1.88 ± 0.12 d	52.5 ± 8.0 bc	0.62 ± 0.04 bc	35.0 ± 7.6 a	44.2 ± 1.6	0.44
2	large	40	3.50 ± 0.14 a	82.5 ± 6.1 ab	0.94 ± 0.04 a	50.0 ± 8.0 a	44.6 ± 1.8	0.58
3	large	40	3.15 ± 0.10 b	65.0 ± 7.6 abc	0.92 ± 0.04 a	27.5 ± 7.1 a	43.5 ± 2.2	1.20
12	large	20	2.40 ± 0.22 c	36.8 ± 11.4 c	0.74 ± 0.07 abc	47.4 ± 22.1 a	33.8 ± 13.3	0.67
13	large	40	3.04 ± 0.10 b	75.0 ± 6.9 ab	0.82 ± 0.04 ab	37.5 ± 7.8 a	43.2 ± 1.5	0.88
14	large	40	2.22 ± 0.10 cd	92.5 ± 4.2 a	0.71 ± 0.03 bc	35.0 ± 7.6 a	45.0 ± 0.9	1.33
15	large	20	1.90 ± 0.11 d	50.0 ± 11.5 bc	0.66 ± 0.06 bc	35.0 ± 10.9 a	46.5 ± 1.9	1.33
17	large	20	1.39 ± 0.20 e	75.0 ± 9.9 ab	0.59 ± 0.08 c	30.0 ± 10.5 a	46.6 ± 2.9	0.50

[1]Means ± SEM.
[2]Means within a column followed by the same letter are not significantly different (P > 0.05; SNK).
[3]Small, 100-ml cup with 100 g diet for 3 weeks prior to entry into sugarcane. Large, 500-ml cup with 200 g diet for 5 weeks prior to entry into sugarcane.

chosen for further evaluation in replicated tests based on larval and adult biomass and survival. Diets containing soybean oil (diets 4-8, and 10) resulted in high mortality of *R. cruentatus* larvae. The published diet (Rahalkar et al. 1978, 1985) was successful for culture of *R. cruentatus* larvae but was eliminated because of difficulty in obtaining coconut cake. Finely chopped field (*S. palmetto*, diet 1) and laboratory (pineapple, diet 11) hosts were unacceptable for growth, development, and survival of *R. cruentatus* larvae when brewers yeast, sucrose, molasses and salts were omitted (Table 2). However, when brewers yeast was added to chopped pineapple (diet 16), the diet was acceptable for larval growth, development, and survival (Table 2). This suggests that yeasts or some other undefined ingredients present in rotting host tissues are an important component in *R. cruentatus* nutrition. The importance of microbes is not surprising considering that many members of the Rhynchophorinae are associated with fermenting and rotting host tissues (Vaurie 1971). Further modification of the pineapple and brewers yeast diet (16) to include sucrose, molasses, oats, and salts with a reduction in the amount of pineapple (diet 2) resulted in improved larval performance. Supplemental cholesterol (diets 3, 4, 5, 6, 13, 14, 15, and 17) or the addition of egg yolk did not improve larval growth.

Weevils cultured by the methods of Giblin-Davis et al. (1989) had a mean generation time of 78-79 days, with 47-48 days from placement of larvae in sugarcane to adult emergence. Mean time to adult emergence in this study ranged from 33.8 ± 13.3 days (diet 12, large cup) to 46.6 ± 2.9 days (diet 17, large cup). Adults (sexes combined) emerging from field-collected cocoons average 1.14 g (Giblin-Davis et al. 1989), while adults cultured by the pineapple/sugarcane method of Giblin-Davis et al. (1989) had a mean biomass of 0.72 g (males) and 0.75 g (females). Weevils cultured by the pineapple/sugarcane method of Giblin-Davis et al. (1989) for over two years had a mean adult biomass of 0.89 g ($n = 704$). Biomass of *R. cruentatus* adults cultured on artificial diets in this study (refined test) ranged from 0.59 g (diet 17, large cup) to 0.94 g (diet 2, large cup) (Table 3). These comparisons suggest that *R. cruentatus* cultured on artificial diets in this study are of comparable biomass to weevils reared using the pineapple/sugarcane method of Giblin-Davis et al. (1989), but adults are slightly smaller than those collected from the field.

Sugarcane appears to contribute very little to the continued growth of mature *R. cruentatus* larvae (Giblin-Davis et al. 1989). Using data from two years of culture on pineapple and sugarcane, we determined that the biomass of sugarcane pieces used for pupation is not correlated to biomass of adults (unpublished data). However, larval biomass is positively correlated with adult biomass (unpublished data). The biological significance of adult biomass and its importance in the fitness of *R. cruentatus* has yet to be explored. Weevils fed artificial diets in large cups had significantly greater larval and adult biomass than weevils reared in small cups. However, larvae were in culture for two weeks longer in the large cups. Thus, if larger larvae or adults are required, larvae should be cultured for longer periods of time with an increased amount of diet. The best diet tested for culture of *R. cruentatus* was diet 2 (supplemented pineapple and oats), in both sizes of diet cups (Table 3). The simplest diet tested that provided larval growth was diet 16, a mixture of canned pineapple and brewers yeast. Further testing indicated that by using a combination of 300 g of diet 3 in 500-ml cups and sugarcane, we were able to culture *R. palmarum* larvae to the adult stage ($n = 5$, 6.8 g larval mass, 80% larval survival, 2.5 g adult mass, 40% adult emergence) (unpublished data).

In conclusion, we were able to successfully culture *R. cruentatus* larvae by using readily available ingredients. We suspect that sugarcane is nothing more than a source of fiber from which the cocoon is constructed by *R. cruentatus* larvae. A limiting

factor in the culture of *R. cruentatus* to adults in many geographic locations may be the availability of sugarcane. We have tested alternative fiber sources added directly to 500-ml diet cups after larvae were in culture for 5 weeks but larvae failed to construct cocoons. Pupal induction in *R. cruentatus* needs further study but appears to require warm temperatures, low relative humidity, and a high fiber substrate.

The culture of *R. cruentatus* on artificial diets has several interesting implications. The use of agar-based diets, although somewhat odorous, has greatly improved working conditions in the rearing facility. In addition, larvae can be reared individually and their history can be documented. This will be important in future studies to determine the vector relationship of *R. cruentatus* with the red ring nematode, *B. cocophilus*. Larvae of *R. cruentatus* are large and would be well suited for use in physiological studies. For example, using the pupae of *R. palmarum* and *Sitophilus oryzae* (L.), Rahbé et al. (1990) identified a new class of hemolymph storage proteins. Finally, although we are aware of no human consumption of *R. cruentatus* larvae in the U.S., larvae of *R. palmarum* (Woodruff 1967), *R. phoenicis* (F.), and *R. ferrugineus* (DeFoliart 1990) are considered delicacies by some. The culture of *R. cruentatus* on artificial diets could be a potential advancement in developing a niche for consumption of our indigenous species by palm weevil gourmets or feeding burrowing owls in captivity (Yosef & Deyrup 1994).

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MORBIDITY OF THE PUPAL STAGE OF THE MEXICAN AND WEST INDIAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) INDUCED BY HOT-WATER IMMERSION IN THE LARVAL STAGE

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ABSTRACT

Heat treatments are used to disinfest fruit exported from regions where *Anastrepha* fruit flies are indigenous. Larvae that survive the heat treatments typically form misshapen puparia. The assumption that all of these puparia will die before eclosion of the adult is shown to be incorrect. Two types of malformed puparia are typically induced by hot-water immersion. A larviform puparium is seldom viable with an eclosion rate of <1% in both the Mexican and West Indian fruit flies. However, a bottle-nosed puparium will produce an adult about 50% of the time. It should be assumed that if any larvae survive treatment to form puparia, some will give rise to adults.

Key Words: *Anastrepha*, morbidity, puparial malformations, heat treatments, quarantine, Tephritidae.

RESUMEN

Los tratamientos con agua caliente se utilizan en regiones donde se encuentran moscas del género *Anastrepha* para desinfestar las frutas que van a ser exportadas. Las larvas que sobreviven los tratamientos de calor producen pupas malformadas. Se demostró que la suposición de que todas las pupas mueren antes de la eclosión del adulto es incorrecta. Dos tipos de puparios malformados se pueden inducir con el tratamiento de agua caliente: larviformes y con nariz en forma de botella. Los puparios larviformes raramente son viables, con una tasa de eclosión menor del 1% tanto en la mosca mexicana como en la mosca de las frutas de las Indias Occidentales. Sin embargo, las pupas con nariz en forma de botella producen adultos en aproximadamente el 50% de los casos. Debe asumirse que si algunas larvas sobreviven al tratamiento y forman puparios también darán lugar a adultos.

The Mexican fruit fly, *Anastrepha ludens* (Loew), and the West Indian fruit fly, *Anastrepha obliqua* (Macquart), are quarantined insect pests. Importation of host fruits to the United States from countries where these flies are indigenous requires a disinfestation treatment. For example, Mexican mangoes are given a hot-water immersion for 1 h at 46°C, a treatment reported by Sharp (1988) to disinfest the fruit of tephritid immatures. USDA-APHIS quarantine restrictions mandate a treatment which will cause mortality of the infesting insects at the Probit 9 level, equivalent to 99.9968% mortality (Baker 1939; see also Chew 1994, Robertson et al. 1994, Shannon 1994, for recent discussion).

In reviewing the published studies on hot-water immersion treatments of various combinations of host fruits and infesting insects, we noted that in many cases, malformed puparia were counted in the mortality figures (Sharp 1986; Sharp et al.

1989a,b,c; Sharp & Hallman 1992). We were concerned that scoring malformed puparia as dead insects without supporting data might include an undefined risk of disinfestation failure.

We, therefore, undertook experiments emphasizing marginally sublethal temperature levels designed to produce large numbers of malformed puparia. The purpose of these experiments was to identify the kinds of malformations associated with hot-water immersion treatments and to quantify the frequency of these malformations and their specific mortality rates.

MATERIALS AND METHODS

All experiments were conducted at the USDA Subtropical Agriculture Research Laboratory in Weslaco, Texas. Ten-day-old, third-instar larvae of *A. ludens* and *A. obliqua* were obtained from colonies of these insects maintained at this laboratory using rearing procedures described by Rhode (1957) and Rhode & Spishakoff (1965) for the Mexican fruit fly, and by Mangan & Ingle (1992) for the West Indian fruit fly. Under laboratory conditions at room temperature, 10-day-old larvae are sufficiently mature to pupariate and metamorphose successfully to adults. The colonies of both species were founded with material originating in Mexico, the *A. obliqua* colony dating from 1987 and the *A. ludens* colony originating in 1953.

All larvae, except controls, were immersed in a circulating water bath controlled to ± 0.02°C by a computer driven temperature control system. The computer software program "Water Troll" was developed by J. J. Gaffney, USDA-ARS, Gainesville, FL. The water baths consisted of a series of four 11-liter capacity stainless steel water banks; each equipped with an electric stirrer motor with shaft and propeller, a 1,000 Watt electrical resistance heater and a Hart 1006 precision thermometer. The larvae were free within a 150 cc capacity, organdy mesh basket.

The experimental temperatures used were 38, 40, 41, 42, 43 and 44°C. The water baths were brought to the selected temperature before immersion of the larvae, and all immersions were for a duration of 1 h. The number of replicates performed varied among temperatures and between species because of the difference in the numbers of malformed puparia produced at the different temperatures and the necessity of using those temperatures which produced the most malformations. Lower temperatures which produced few malformations were replicated as a check against the possibility that the malformations could have been caused by immersion in water alone. With *A. ludens* there were eight replicates from two rearing batches at 38°C; five replicates from five rearing batches at 40°C; nine replicates from six rearing batches at 41°C; 16 replicates from four rearing batches at 42°C; 23 replicates from eight rearing batches at 43°C, and six replicates from three rearing batches at 44°C. With *A. obliqua* there were 12 replicates from three rearing batches at 38°C, five replicates from five rearing batches at 40°C; 59 replicates from 20 rearing batches at 41°C; 12 replicates from three rearing batches at 42°C; 14 replicates from five rearing batches at 43°C, and four replicates from one rearing batch at 44°C. The numbers of larvae varied among replicates because of the necessity to avoid unnecessary handling and possibly detrimental effects to the larvae prior to pupariation. Thus, batches of larvae were tested as they became available and the precise numbers tested were counted after treatment during the immobile puparial stage. The mean treatment size was 289.6 larvae per replicate with a total of 61 replicates for *A. ludens*, and 154.7 larvae per replicate with a total of 106 replicates for *A. obliqua*.

Immediately following the hot-water immersion, both treated and untreated control larvae were transferred to 10 cm wide, 250 cc capacity, plastic containers with

screened lids. The containers were half-filled with clean, slightly moistened vermiculite (the pupariation medium) and held in an incubator at 25 ± 2°C temperature, 75 ± 5% humidity and 12:12 L:D cycle. After 3-4 days the puparia were sifted from the pupariation medium and scored as normal or malformed and separated. Dead larvae were counted and removed. The puparia were then returned to the slightly moistened vermiculite, placed in the incubator and held for emergence. After adult eclosion, the puparia were examined to determine the percent eclosion for each type of malformation.

In order to determine the viability of the adults from the malformed puparia, some of the emergent flies were held and tested for fertility. Adults (males and females) from eleven replicates each of *A. ludens* and *A. obliqua* were held in screen cages at 25°C and ambient light cycle with a diet of sugar and hydrolyzed yeast until an age of 10 days. The treated, but normal, puparia were segregated from the malformed puparia. Thus, males from malformed puparia mated with females from malformed puparia, and males from treated, but normal, puparia mated with the corresponding females. Untreated controls from the same rearing batches were also tested.When the flies were ten days old, surviving females (up to 10 if available) from each test group were placed in cages with an artificial gel oviposition medium and left overnight. The numbers of eggs oviposited were counted on the following day. These eggs were held and re-examined three days later to determine the percent hatch.

Differences in mean mortality between treatment and control groups were tested using the t-test for paired comparisons (Sokal & Rohlf 1973). The relationship between temperature and mortality and between temperature and frequency of malformation was computed with the product moment correlation coefficient (Sokal & Rohlf 1973). The mean eclosion rate from normal vs. malformed puparia was tested using single classification analysis of variance (Model I ANOVA) (Sokal & Rohlf 1973). The probability value *P* for each *F*, *t* and *r* statistic was calculated with the software program Speakeasy (Speakeasy Computing 1987).

Results and Discussion

A one-h exposure to a water bath at 44°C resulted in 100% total mortality in four replicates of *A. obliqua* and 99.8 ± 0.57% mean (±SE) mortality in six replicates of *A. ludens*. A total of two adults were produced from 1,132 *A. ludens* larvae tested, and none from the 624 *A. obliqua* larvae tested. However, at 43°C, mean mortality was 79.2 ± 12.5% in 23 replicates of *A. ludens* (n = 6,105) (Table 1). *A. obliqua* was more susceptible to these temperatures; at 43°C only one adult was produced from 2,619 treated larvae (14 replicates), a mean mortality rate of 99.96 ± 0.15%. At 42°C, only 5 adults were produced from 2,480 treated larvae, a mean mortality rate of 99.8 ± 0.35% (12 replicates). At 41°C, however, there was appreciable survival to the adult stage, with a reduction in mean mortality to 75.6 ± 12.0% (58 replicates) in *A. obliqua* (Table 2).

Significant mortality was produced at all water temperatures tested except the lowest. At the lowest test temperature, 38°C, there was 69.6 ± 8.4% mean survival in 12 replicates of *A. obliqua* larvae (n = 2,526), whereas 85.9% of the controls from all tests produced adults (Table 1.) The difference in mean survival, defined as the percentage of adults emerging from puparia, was statistically significant using a pair-wise t-test (t = 3.98; df = 11; P = .001). But, for *A. ludens*, the difference in survival at the low temperature was much less. When subjected to 38°C for 1 h, mean survival was 84.5 ± 13.6%, compared to a mean survival of 84.5 ± 12.9% in controls.

TABLE 1. NUMBERS OF DEAD LARVAE, MALFORMED PUPARIA, NORMAL PUPARIA AND ECLOSED ADULTS AFTER HEAT TREATMENTS ON THE 3RD INSTAR LARVAE OF *ANASTREPHA LUDENS*.

			Normal		Malformed			
Test Temp	Total n	Dead Larvae	Total Pupae	Adults Eclosed	Bottle-nose	Larvi-form	Peanut Pupae	Adults Eclosed
C	6059	110	5917	5500	25	3	4	13
38°	2362	106	2172	1913	82	2	0	44
40°	1154	68	1049	863	31	2	4	25
41°	1911	258	1488	1085	164	0	1	72
42°	5017	530	3834	3576	576	72	5	447
43°	6105	2668	2281	1090	924	228	4	197
44°	1132	1104	1	0	4	23	0	2

The difference in survival was not significant with a pair-wise t-test ($t = 0.18$; $df = 7$; $P = 0.43$).

Larval Mortality

Most of the mortality produced by the hot-water treatment was to the larval stage: 57% of total deaths in *A. ludens* and 55% of total deaths in *A. obliqua*. Larvae immersed in hot water stretched to their full length and became immobile. Surviving larvae recovered mobility within 1-2 h and most pupariated eventually. Those which did not recover mobility turned black and shriveled within 1-2 days.

At the lowest test temperature, 38°C, there was a mean mortality of 11.4 ± 1.9% in *A. obliqua* larvae and 4.2 ± 2.9% in *A. ludens* larvae. However, even this low level of mortality was significantly greater than for the untreated controls in which failure to pupariate was 3.5 ± 2.3%. Using a pair-wise t-test to compare larval mortality at

TABLE 2. NUMBERS OF DEAD LARVAE, MALFORMED PUPARIA, NORMAL PUPARIA AND ECLOSED ADULTS AFTER HEAT TREATMENTS ON THE 3RD INSTAR LARVAE OF *ANASTREPHA OBLIQUA*.

			Normal		Malformed			
Test Temp	Total n	Dead Larvae	Total Pupae	Adults Eclosed	Bottle-nose	Larvi-form	Peanut Pupae	Adults Eclosed
C	3455	73	3291	2945	73	15	6	24
38°	2526	285	2078	1704	152	7	4	51
40°	2454	176	2058	1354	179	13	4	81
41°	7831	1846	2193	1001	2898	891	3	765
42°	2480	1935	225	3	204	116	0	2
43°	2619	2590	1	1	14	14	0	0
44°	624	624	0	0	0	0	0	0

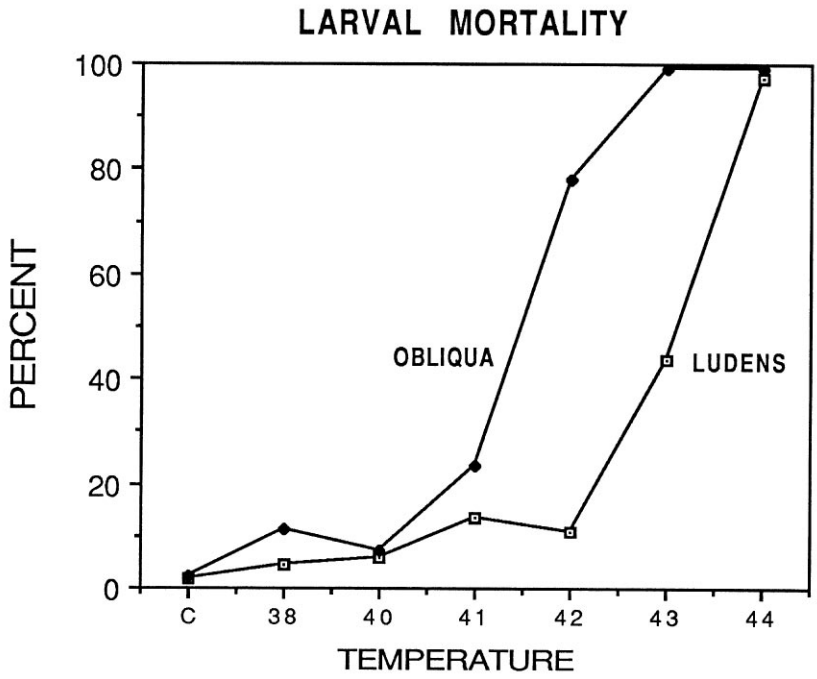

Fig. 1. Mortality in late third-instar larvae of *Anastrepha ludens* and *A. obliqua* following one h exposure in hot-water bath at 38-44°C, and non-exposed controls (c).

38°C, the t-value for *A. ludens* was 7.19 ($df = 7$, $P = 8.5^{e-5}$); and for *A. obliqua* $t = 7.41$ ($df = 11$, $P = 6.73^{e-6}$). Larval mortality rate increased sharply at 1 h exposures to 42°C for *A. obliqua* and at 43°C for *A. ludens* (Fig. 1).

Pupal Mortality

There was a 100% failure of larvae to pupariate at only one test temperature, 44°C, and for only *A. obliqua*. Some adults enclosed in all cases in which at least some larvae pupariated. Pupal mortality was significantly correlated with temperature in both species. The correlation coefficient (r) between temperature and mortality in *A. obliqua* was 0.94 ($P = .0026$). Mortality rose sharply at 41°C to 69.5 ± 12.2% and at test temperatures of 42°C, mean mortality was 99.2 ± 1.5% in this species. For *A. ludens* the correlation between temperature and pupal mortality was not as rigid but still significant ($r = 0.785$, $P = .032$). The test temperatures did not induce high pupal mortality in *A. ludens* until 43°C and above (Fig. 2).

Puparial Malformations

Pupariation in cyclorrhaphous Diptera is a four-step process (Zdarek & Frankel 1972, 1987). First, the anterior segments invert. Second, flexion of the integumental musculature constricts the body into a barrel-shape. Third, the cuticle shrinks (mainly by dehydration), and fourth, the cuticle becomes sclerotized through mela-

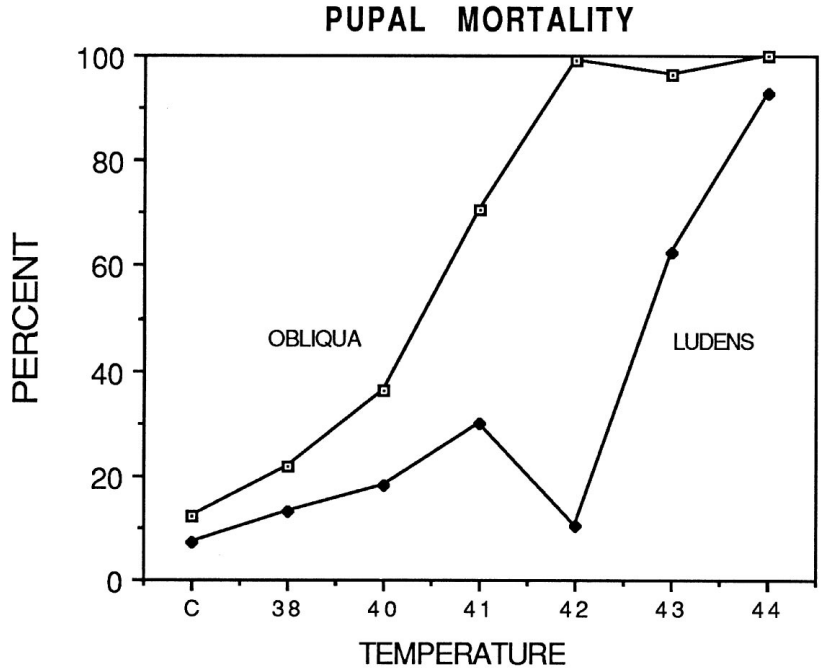

Fig. 2. Mortality in the pupal stages of *Anastrepha ludens* and *A. obliqua* from larvae subjected to hot-water immersion, and non-treated control larvae (c).

nization. Puparial malformations can be explained as dysfunctions of one or more of these mechanisms (Thomas & Mangan 1990).

Three types of malformations were observed in the test groups of puparia. The rarest form, a peanut-shaped puparium, was found in both treated and untreated groups. This malformation was characterized by a mild constriction in the middle segments. Its immediate cause was not determined but did not appear to be induced by, or associated with, hot-water immersion. A total of 17 cases were seen in *A. obliqua* (6 among the controls); and 18 cases in *A. ludens* (4 among controls). From the 35 peanut-shaped puparia, 15 adults emerged. These numbers were too small to treat statistically.

Larviform (Fig. 3a) and bottlenose (Fig. 3b) puparia were found to be associated with hot-water immersed larvae in both species. The larviform malformation was characterized by a failure to constrict into a typical barrel shape, and a failure of the head segments to invert. Of the 1,056 larviform puparia of *A. obliqua*, none produced an adult (100% mortality). However, of the 330 larviform puparia induced in *A. ludens*, two adults eclosed (99.1% total mortality). Both were from the same treatment cohort subjected to 42°C. There was a significant correlation between temperature and the percent frequency of larviform puparia in *A. obliqua* ($r = 0.88$, $P = .0245$). For *A. ludens*, the correlation was not significant at the 95% confidence level ($r = 0.64$, $P = .086$). The lower mathematical correlation seemed to result from the abrupt increase in this type of malformation at the highest treatment temperature (Fig. 4), as opposed to the linear increase in frequency seen in *A. obliqua* (Fig. 5).

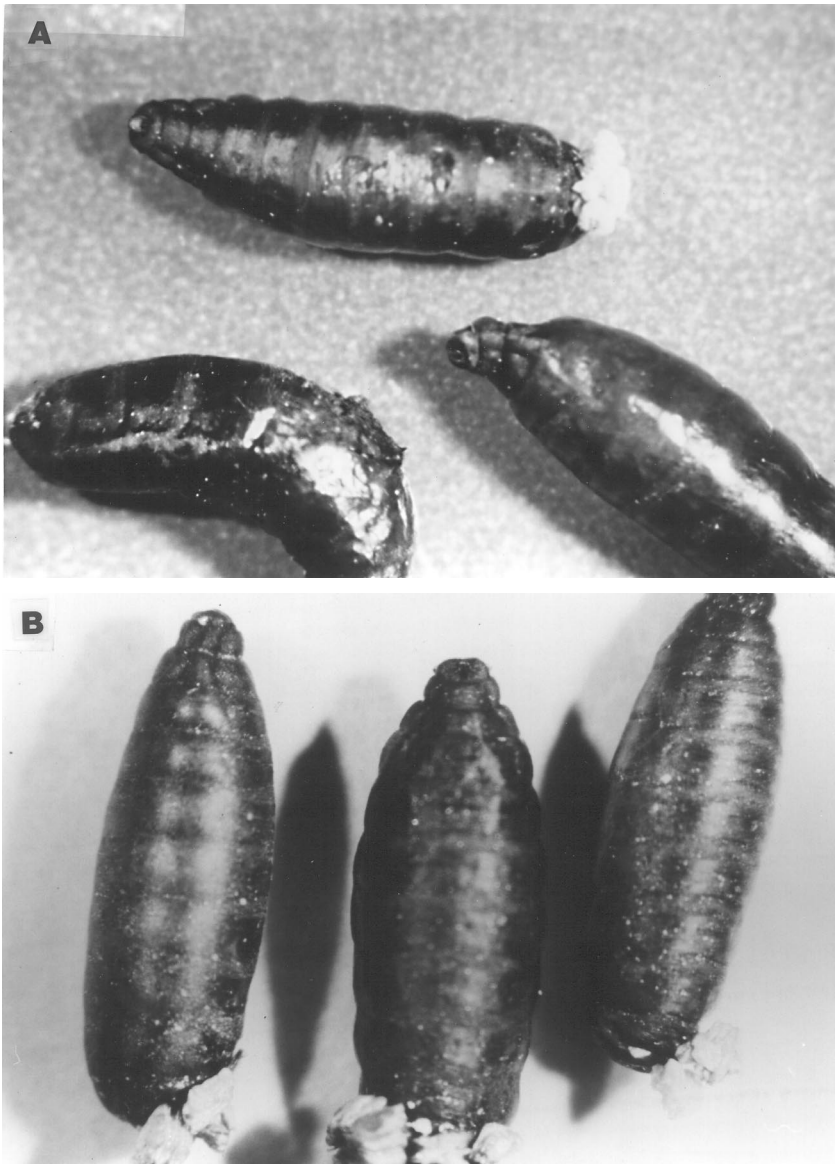

Fig. 3. Puparial malformations in *Anastrepha ludens* and *A. obliqua*: subjected to hot-water immersion. (a) larviform puparia; (b) bottlenose puparia.

In the bottlenose malformation, the anterior-most segment of the puparium is abnormally constricted, but otherwise morphologically asymptomatic (Fig. 3b). The frequency of the bottlenose malformation was significantly correlated with temperature in both species: $r = 0.77$ ($P = .037$) for *A. ludens*; $r = 0.84$ ($P = .038$) for *A. obliqua*. The greatest numbers of bottlenose puparia were produced at 40-41°C (*A. obliqua*) and

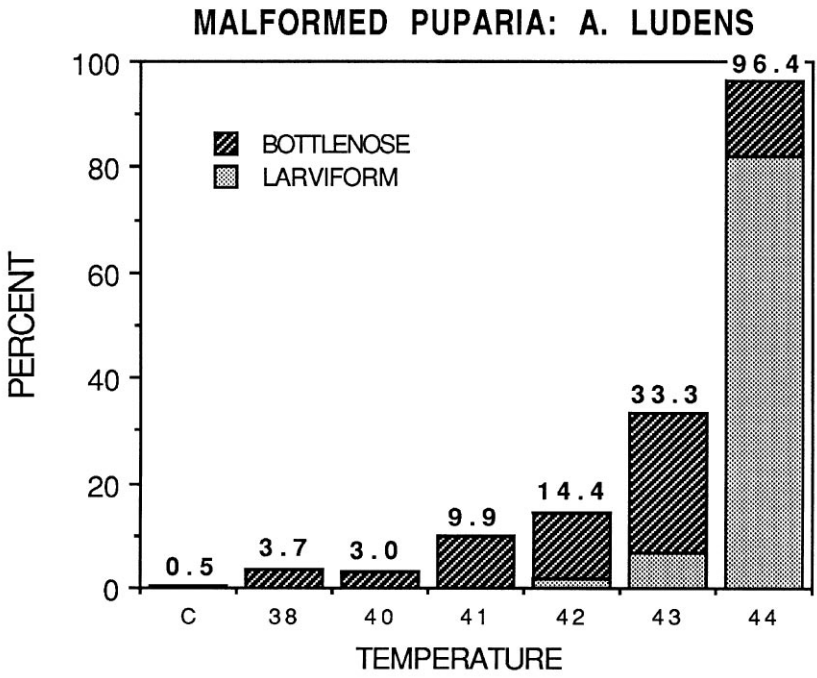

Fig. 4. Frequency of larviform or bottlenosed puparia following exposure of late third-instar *A. ludens* larvae to one-h hot-water bath at 38-44°C, and non-exposed controls (c).

41-42°C (*A. ludens*). The survival of the pupal stage to eclosion as an adult was significantly less in the bottlenose puparia compared to the normal puparia at each test temperature for both species. With *A. obliqua*, eclosion rate at 40°C was 75.9 ± 14.5% for the normal puparia, but only 51.9 ± 15.1% for the bottle-nose puparia. The difference was significant tested by ANOVA (F = 9.19; df = 1, 12; P = .01). Eclosion rate at 41°C was 37.4 ± 13.5% for the normal puparia, but only 25.4 ± 8.9% for the bottlenose puparia, a significant difference (F = 7.67; df = 1, 26; P = .01). With *A. ludens*, adult eclosion rate at 41°C was 73.4 ± 14.2% for the normal puparia, but only 50.5 ± 20.4% for the bottlenose puparia (F = 7.59; df = 1, 16; P = .01). At 42°C the adult eclosion rate from the normal puparia was 92.7 ± 5.3%. In comparison, the eclosion rate was lower, 78.7 ± 12.7% for the bottlenose puparia, but the difference was not significant at the 95% confidence limit with ANOVA (F = 4.21; df = 1, 6; P = .09).

Morbidity

Morbidity is the proportion of sick individuals in a population (Lapedes 1976). Hot-water immersion of late third-instar larvae at sublethal temperatures resulted in significant numbers of individuals which turned black and failed to pupariate or which formed misshapen puparia in both *A. ludens* (Fig. 6) and *A. obliqua* (Fig. 7). Adults failed to eclose from most of the malformed puparia. However, the results of this study strongly suggested that morbidity can be equated with mortality only if the

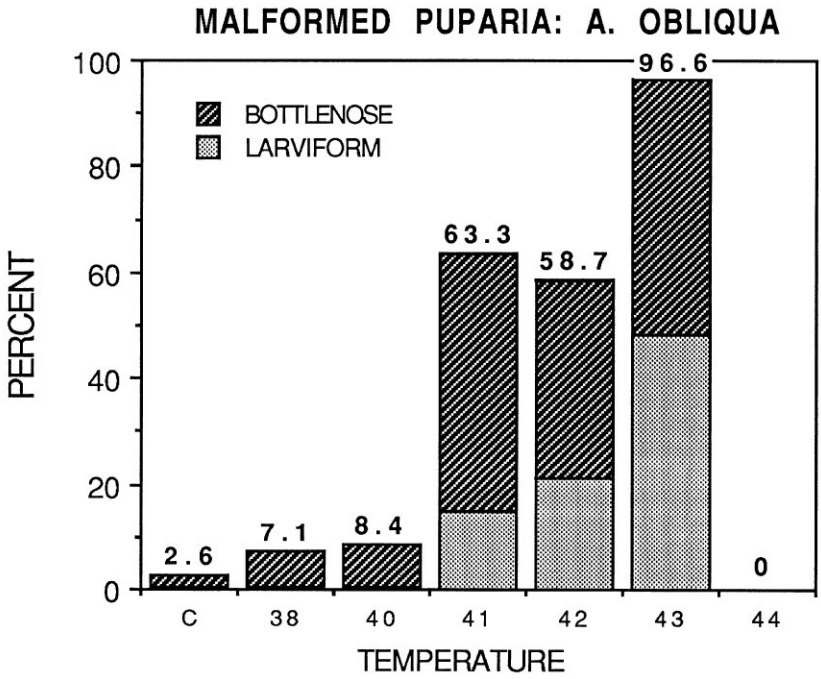

Fig. 5. Frequency of larviform or bottlenosed puparia following exposure of late third-instar *A. obliqua* larvae to one-h hot-water bath at 38-44°C, and non-exposed controls (c).

puparia are scored for the class of malformation. The larviform type of malformation was lethal at a rate in excess of 99%. Conversely, a high percentage of the bottlenose malformations are viable. In these experimental treatments, it was found that some of the larvae that survived to pupariate eventually reached the adult stage, even under conditions in which the mortality of the larval stages was greater than 99% and all of the puparia were malformed.

Followup tests with the adults that survived the hot-water treatments demonstrated that they were capable of attaining reproductive age, mating and ovipositing at normal levels. This was true for adults from treated but normal puparia, as well as for those emerging from malformed puparia. Mature *A. ludens* females (n = 66) from normal puparia oviposited a mean of 26.6 ± 25.0 eggs, of which 75.9% hatched. Females (n = 69) from bottlenosed puparia oviposited a mean of 14.9 ± 14.5 eggs, of which 65.3% hatched. Control females (n = 35) laid a mean of 13.8 ± 10.8 eggs, of which 77.5% hatched. Mature *A. obliqua* females from normal puparia oviposited a mean of 13.5 ± 10.8 eggs (n = 66), of which 61.2% hatched. Females (n = 65) from bottlenosed puparia oviposited a mean of 8.0 ± 6.9 eggs, of which 64.5% hatched. Control females (n = 67) oviposited a mean of 12.3 ± 11.5 eggs, of which 65.0% hatched.

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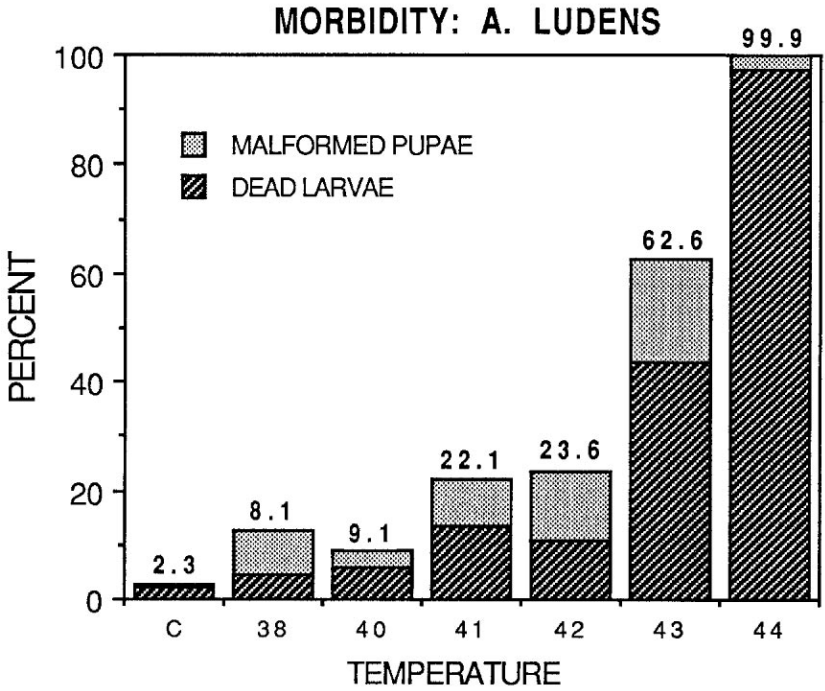

Fig. 6. Morbidity: larval death or puparial malformation following exposure of late third-instar *A. ludens* larvae to one-h hot-water bath at 38-44°C, and non-exposed controls (c).

the test insects and for data collection. Guy Hallman and Felix Guerrero provided valuable reviews of the manuscript.

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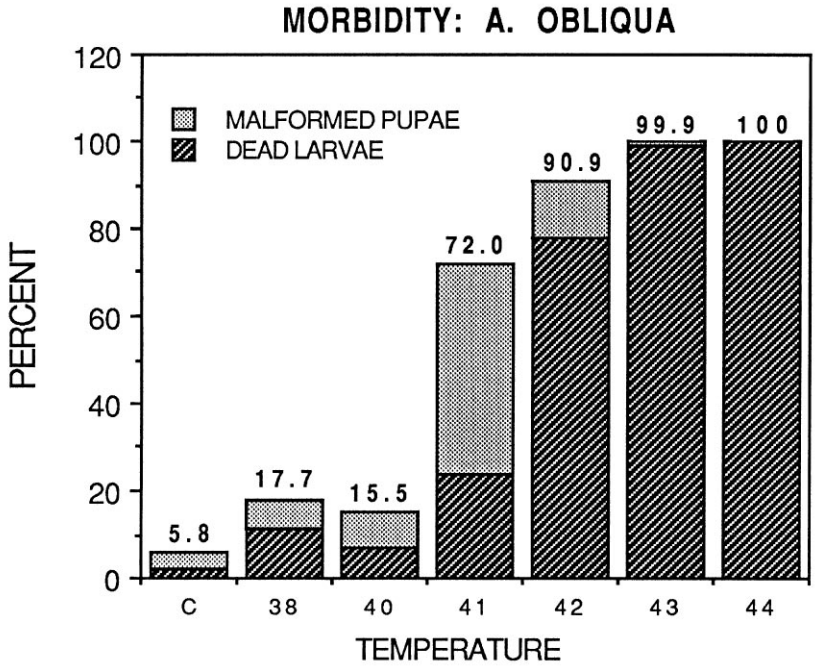

Fig. 7. Morbidity: larval death or puparial malformation following exposure of late third-instar *A. obliqua* larvae to one-h hot-water bath at 38-44°C, and non-exposed controls (c).

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A NEW SPECIES OF *PETREJOIDES* FROM GUATEMALA AND COMMENTS ON *PETREJOIDES MICHOACANAE* (COLEOPTERA: PASSALIDAE)

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ABSTRACT

Petrejoides caralae **New Species** is described from wet lowland forest of eastern Guatemala (450-950 m altitude). The aedeagus of *Petrejoides michoacanae* Schuster is described.

Key Words: Aedeagus, Mexico.

RESUMEN

Se describe *Petrejoides caralae* **Nueva Especie** de bosque húmedo de tierras bajas, del este de Guatemala, entre 450-950 metros de altitud. Se describe el edeago de *Petrejoides michoacanae* Schuster.

Only two of the 18 previously described species of *Petrejoides* are known from below 1000 m altitude (Schuster 1991). Here we describe a new species from a wet, lowland forest of two isolated mountains of eastern Guatemala (Fig. 1). We also comment on the holotype of *Petrejoides michoacanae* Schuster and describe the aedeagus.

Petrejoides caralae Cano & Schuster **NEW SPECIES**

Figures 2, 3

Description

Head: anterior border of labrum concave, anterior angles rounded. Clypeus inclined, short (anterior-posterior), rectangular, anterior border linear slightly arcuate, anterior angles rounded and directed downward, smooth and brilliant. Frontoclypeal suture slightly arcuate. External tubercles distinct, rounded.

Frontal area short, without inner tubercles. Frontal ridges poorly marked. Frontal fossae glabrous or with 1-2 setae. Median frontal structure of "falsus" type (see Reyes-

Fig. 1. Distribution of *Petrejoides caralae* **New Species**. Square = Sierra de Caral, circle = Cerro San Gil. Elevations: 0-500 m = irregular dots, 500-1500 m = regular dots, 1500-2500 m = horizontal lines, >2500 m = vertical lines.

Castillo 1970), center horn short with apex not free, without median longitudinal groove posteriorly; lateral ridges curved slightly forward, with terminal tubercles. Occipital groove well marked, terminating in supraorbital ridges.

Anterior 1/2 of supraorbital ridge bituberculate, posterior 1/2 not bifurcate. Anterior cephalic angle rounded. Canthus not swollen distally, apex rounded, not, or barely, reaching lateral eye margin. Dorsal width of an eye 1/8 head width.

Ligula between insertions of labial palps wide, convex or slightly convex. Lateral lobes of mentum with anterior external border rounded, whole surface punctate and pubescent, lateral border straight. Medial basal mentum bare without punctures, anterior border slightly biconvex. Hypostomal process narrow without lateral depression. Infraocular ridge indistinct, punctate and pubescent.

Mandible with only 2 apical teeth. Mandibular tooth occupies more than 1/2 mandible length. Internal teeth bifid.

Thorax: Lateral fossa of pronotum without punctations, 0-2 other punctations on lateral pronotum outside fossa. Arcuate (3 specimens) or linear (1 specimen) scars dorsally on posterior 1/3 of pronotum, 1 on each side. Pronotum with marginal groove very narrow; anterior angles rounded. Prosternum rhomboidal with posterior apex truncate.

Mesosternum mostly brilliant throughout, narrow rugose line bordering mesepisternum, without or with a few punctations, with a few hairs. Mesosternal groove lightly present. Mesepisternum not rugose, brilliant. Mesepimeron hairy.

Metasternum anterior angles pubescent. Lateroposterior sides of disk delimited by 11-38 punctations on each side; marginal fossa wide, very pubescent.

Anterior elytral profile convex; elytral striations marked uniformly with small, round, light punctations, somewhat heavier in lateral striations; junction of striations 1 and 10 with many extra punctations forming a double line, with many fine hairs.

Wings: as in *Petrejoides recticornis* (Burmeister), not reduced (see Fig. 5 in Castillo & Reyes-Castillo (1984)).

Legs: Femur I with anterior-ventral groove almost absent or slightly marked. Tibia II with long dorsal ridge.

Abdomen: Marginal groove complete around last sternite.

Aedeagus: Median lobe large and globose, with many pigment punctations. Ventral view: parameres and basal piece not completely separated by a distinct suture, the tegmen is narrower at the base than the apex. Dorsal view: Basal piece small, all the way round.

Dimensions (mm). Total length, mandibles to tip of elytra 33-34.2, $\bar{x}$ = 33.5, males 33-34.2, $\bar{x}$ = 33.5, female 33.6; elytral length 18.4-18.7, $\bar{x}$ = 18.6; pronotal length 8.1-8.6, $\bar{x}$ = 8.4; pronotal width 10.8-11.0, $\bar{x}$ = 10.9; humeral width 9.6-10.2, $\bar{x}$ = 9.9; head width 7.4-7.7, $\bar{x}$ = 7.5; aedeagal length 3.6.

Material Examined

Four whole specimens, including one teneral female, two almost black males and one black old male.

Type Material

Holotype male, **Guatemala**, Izabal Dept., Morales, Sierra de Caral, altitude 450 m., 28-X-1992, J. Monzón collector.

Paratypes: Two from same location and collection data as holotype; one from Izabal Dept., Puerto Barrios, Cerro San Gil, 950 m., VI-1993, J. Monzón.

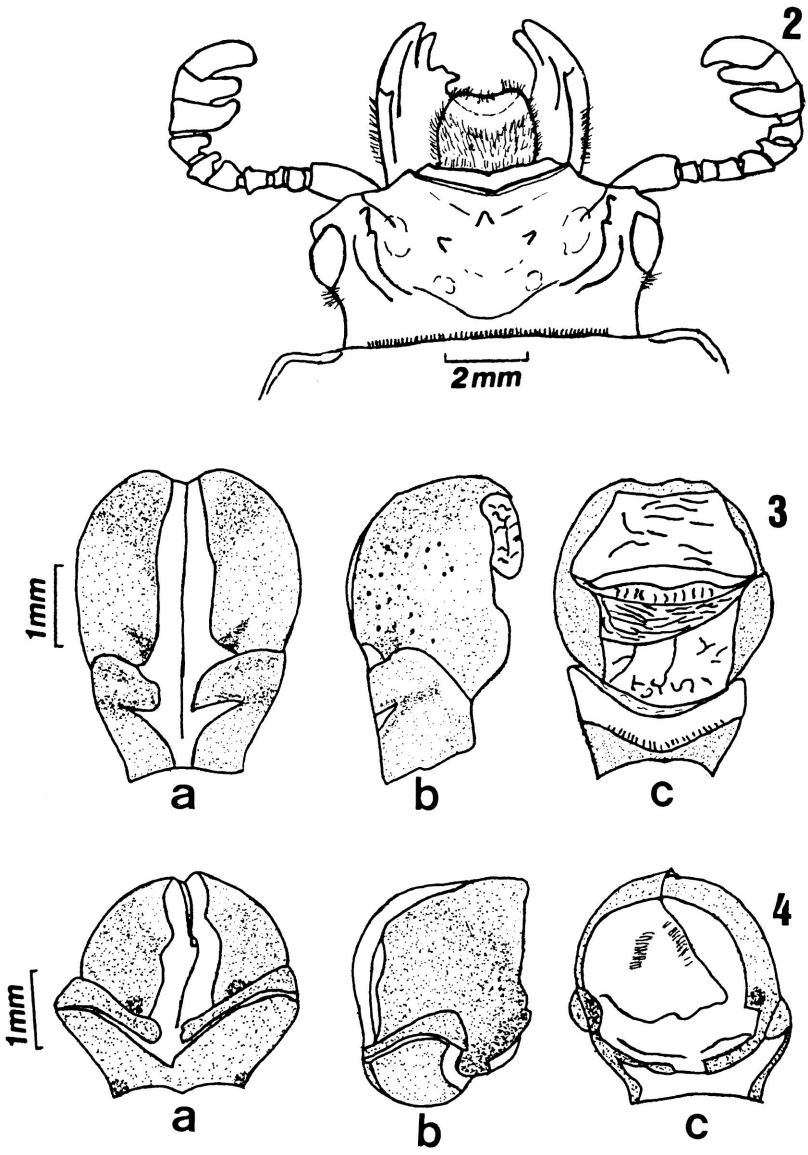
Types at present are in the Arthropod Collection of the Universidad del Valle de Guatemala. We plan to deposit paratypes in the collection of the Instituto de Ecología, Xalapa, Mexico and in the Natural History Museum of Paris.

Etymology

Refers to the mountain from which 3 of the 4 specimens are known, Sierra de Caral, Guatemala.

Distribution

Known from a wet, lowland forest of two mountains of eastern Guatemala, the Sierra de Caral near the Guatemalan-Honduran border, and Cerro San Gil near Puerto Barrios (Fig. 1).



Figs. 2-4. 2 - *Petrejoides caralae* **New Species** dorsal view of head, 3 - *Petrejoides caralae* **New species**: 3a, aedeagus, ventral view; 3b, aedeagus, lateral view; 3c, aedeagus, dorsal view. 4 - *Petrejoides michoacanae* Schuster: 4a, aedeagus, ventral view; 4b, aedeagus, lateral view; 4c, aedeagus, dorsal view.

At present, apparently no gene flow occurs between these two areas. Sierra de Caral and Cerro San Gil are separated by the wide, lowland valley of the Motagua

river. This valley could have been easily crossed during glacial times when montane forests were lower (MacVean & Schuster 1981).

Other species found at the collection sites in the Sierra de Caral include: *Verres corticicola* (Truqui), *Passalus jansoni* (Bates) and *Passalus punctiger* Lepeletier and Serville. On Cerro San Gil, they include: *Proculus opacipennis* (Thompson), *Popilius eclipticus* (Truqui), *Passalus caelatus* Erichson, *Odontotaenius striatopunctatus* (Perch.) and *Paxillus leachi* MacLeay.

Affinities

P. caralae is the largest species of *Petrejoides*. It seems most related to *P. michoacanae* Schuster. These especies are in the "recticornis" group of Castillo & Reyes-Castillo (1984). *P. caralae* can be differentiated from other *Petrejoides* by the following modification in the key of Schuster (1991):

8(7') Mandible with 2 apical teeth, pronotum with a pair of dorsal scars posteriorly; frontal fossae glabrous or with 1-2 setae, metasternum with lateral fossae wide, juncture of elytral striae 1-10 with many fine hairs .... *Petrejoides caralae* **New species**

8' Mandible with 3 apical teeth. Pronotum without dorsal scars posteriorly; frontal fossae pubescent; metasternum with lateral fossae narrow, junction of elytral estriae 1-10 glabrous .....*Petrejoides michoacanae* Schuster

Petrejoides michoacanae Schuster

Schuster (1991) describes this species based on one specimen from Michoacán, México. On reviewing the holotype, we noted that the humeral width is 9.9 mm, not 6.3 mm, and it is male. The aedeagal description is as follows (Fig. 4):

Ventral view: Aedeagus asymmetrical, almost as long as wide, 2.68 mm long. Median lobe almost as long as tegmen. Tegmen divided, narrower at the base than at the apex; lateral lobes more short than basal piece. Dorsal view: Median lobe and basal piece with the sclerotized area lightly visible.

Acknowledgments

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MOVEMENT IN A ZAPROCHILINE KATYDID (ORTHOPTERA: TETTIGONIIDAE): SEX-SPECIFIC RESPONSE TO FOOD PLANT DISTRIBUTION

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ABSTRACT

Movement in relation to local food levels was compared between the sexes of the zaprochiline katydid *Kawanaphila nartee* Rentz. Adults of both sexes were marked, and their departure rates were estimated (1) early in the season for two plots having high vs. low food density and (2) throughout the season for a single plot with changing food levels. Based on the between-plot comparison, movement of males was independent of food density, whereas females were less likely to leave the rich patch than the poor one. The within-plot comparisons revealed that, despite large changes in food levels, male departure rates did not vary over the season. Females showed increased movement over the season and by the end of the study had a departure rate similar to that of males.

Key Words: Spacing, mate searching, Australia.

RESUMEN

Se comparó el movimiento de los machos y hembras de *Kawanaphila nartee* Rentz (Tettigonidae: Zaprochiliinae) en relación con niveles locales de alimento. Adultos de ambos sexos fueron marcados y se calculó la frecuencia de salida (1) en dos parcelas con densidad de alimento alta y baja al comienzo de la temporada y (2) en una sola parcela con niveles de alimento variables a lo largo de la temporada. La comparación entre las parcelas indicó que el movimiento de los machos era independiente de la densidad de alimento mientras que las hembras fueron menos propensas a salir de la parcela con alta densidad. Las comparaciones dentro de cada parcela revelaron que, a pesar de los grandes cambios en los niveles de alimento, la salida de los machos no varió a lo largo de la temporada. Las hembras mostraron incremento de movimiento a lo largo de la temporada y al final del estudio tuvieron una tasa de salida similar a la de los machos.

Males of many insect species encounter and mate with females at resources, such as food or oviposition substrate, that are critical to female reproductive success (Thornhill & Alcock 1983). In many of these species, males aggressively defend territories, and their mating success is directly related to the quality of resources controlled (McLain 1986; Villalobos & Shelly 1991). However, in other species, males do not hold territories and exhibit exploitative (or scramble) competition for mates at re-

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source sites (Banks & Thompson 1985; Hafernik & Garrison 1986). Theoretically, these males are expected to have an ideal free distribution (Fretwell 1972), i.e., to space themselves in such a way that each individual has the same probability of encountering females (Parker 1974).

In an earlier article (Shelly & Bailey 1992) on the Australian katydid *Kawanaphila nartee* Rentz, we reported a difference between the sexes in their distribution relative to the local abundance of the primary foodplant. Censuses of nine plots revealed that female abundance increased with increasing foodplant density, whereas male numbers varied independently of foodplant density. Correspondingly, female:male ratios increased with increasing resource richness in the plots. Because males are not territorial (Bailey & Simmons 1991), the resource-dependent increase in female:male ratio suggested that males had more mating opportunities in rich food patches than small ones, i.e., they were not distributed in an ideal free manner.

Focusing on the same katydid species, we here examine movement tendencies of males and females in response to resource abundance. Specifically, we estimated departure rates of the sexes (1) simultaneously for two plots with high vs. low foodplant density and (2) over the adult season for a single plot whose foodplant density showed marked temporal variation.

Materials and Methods

Study Site and Species

Field work was conducted during August-November 1990 in Kings Park, a large area of bushland in Perth, Western Australia. The park is largely a shrub-grassland with an open canopy dominated by various species of *Banksia* and *Eucalyptus* trees.

The life history, feeding biology, and mating system of the species have been described elsewhere (Gwynne & Bailey 1988; Gwynne & Simmons 1990; Simmons & Bailey 1990; Bailey & Simmons 1991; Rentz 1993), and the following summary derives from these earlier studies. The species is univoltine, with adults sexually active between September-November. The adults, which are flightless, feed exclusively on pollen and nectar. A variety of foodplants are utilized, but kangaroo paws (*Anigozanthos manglesii* Endl.), which flowers from August to October (see Results), is the main food source during the early and middle portions of the adult season. As kangaroo paws decline, the zaprochilines switch their feeding to a species of grasstree (*Xanthorrhoea preissei* Endl.), which flowers from August to November. Grasstrees are far less abundant than kangaroo paws and typically occur as isolated individuals separated by distances of 2-10 m. However, each grasstree produces a large stalk bearing thousands of flowers and thus represents a rich, albeit localized, source of pollen and nectar.

Mating behaviour involves the attraction of females to stationary males calling within the vegetation. Males sing throughout the night, but sexual activity is restricted to the 3-h period immediately following sunset (1900-2200 hours). Males are not territorial and are not physically aggressive toward one another. During mating, the male transfers a large, proteinaceous spermatophore to the female, which she then consumes. Both field observations and experiments reveal a seasonal change in sex roles dependent on food availability. Females are apparently food-limited during the kangaroo paws season, and males are the choosy sex at this time. Conversely, flowering grasstrees provide superabundant food resources, and females are then the discriminating sex. Females appear to mate multiply, though field data on remating frequency are inexact.

Zaprochiline Abundance and Movement

During the kangaroo paw season, movement of zaprochilines was studied in 2 plots, each measuring 10 m by 10 m. The main plot (plot A) had a high density of kangaroo paws and was monitored during 3 different periods corresponding to the early (1-22 September), middle (25 September-16 October) and late (20 October-10 November) parts of the kangaroo paws season. The other plot (plot B) had a much lower density of kangaroo paws and was monitored only during the early part of the kangaroo paws season. Counts of flowering kangaroo paws were made approximately one week after the initial zaprochiline census for a given monitoring period. Plots A and B were separated by a distance of approximately 30 m and contained the same sets of plant species.

During a given monitoring period, we censused a plot every 3 days for a period of three weeks. Censuses were performed between 1900-2200 hours, usually under clear skies, but occasionally during light rain (zaprochilines were still active under such conditions). During a census, we slowly and systematically moved through a plot and attempted to locate all *K. nartee* adults. Calling males produce an ultrasonic signal (Gwynne & Bailey 1988) and were located using a "mini bat detector" (QMC Instruments) set at the average dominant frequency of the call (50 kHz). Females and non-calling males were located visually by scanning vegetation with a head lamp.

During the first 3 censuses of a given monitoring period, we marked all individuals with unique color sequences of enamel paint on their abdomen and released them at their original perch. Marking had no apparent effects on the subsequent behaviour of the insects, and individuals typically resumed calling or feeding within minutes of being handled. In the remaining censuses, we simply noted the presence of marked individuals and counted unmarked ones.

Data on the presence or absence of marked individuals were used to calculate emigration rates for both sexes. The numbers of individuals remaining in the plot through time were log transformed to produce decay curves analogous to survivorship curves used in demographic studies. Pairwise slope comparisons followed the method of Zar (1974). Tests significant at $p=0.005$ were also found to be significant using adjusted critical values of the Bonferroni correction (Kleinbaum et al., 1988).

In examining the presence/absence data, we found that some individuals had a "gap" in their record such that they were not observed on a given census date(s) but were observed on the census dates immediately preceding and following the gap. In almost all cases, the gap consisted of only one census date, and we assumed the individual was either missed or was immediately adjacent to the plot and therefore treated the individual as being present throughout (i.e., operationally, we ignored the gap). However, where the gap consisted of 2 or more consecutive census days, we assumed the individual moved away from the plot and considered the first day of absence to be its departure date.

As the preceding paragraph indicates, we assume that the disappearance of marked individuals from our study plots resulted primarily from emigration and that mortality was of secondary importance. This assumption appears valid, because (1) individuals typically live for several weeks in the laboratory (Simmons & Gwynne 1993), yet the numbers of marked individuals decreased markedly over just a few days (see Results) and (2) compared to the relatively straightforward explanation involving variable food abundance, unusual (and therefore unlikely) patterns in predation intensity would be required to account for the spatial and sexual differences observed in population decay curves (see Results). In addition, because of the rapid disappearance of marked individuals, successive censuses in a plot were not simply repeated estimates for the same local population of insects. Though census data from

different dates were not completely independent (since some individuals were counted on two or more censuses), the high turnover of individuals lessened this statistical problem, and hence inter- and intra-plot comparisons of abundance were considered valid.

During censuses in the early part of the kangaroo paws season, we also marked the position of each individual in plots A and B, respectively, by placing a numbered tag immediately adjacent to the perch. On the day following a census, we recorded the coordinates of these tags (to the nearest 0.1 m) on gridded maps of the study plots. These data were used to investigate spacing patterns of both sexes via analysis of nearest neighbor distances (Clark & Evans 1954).

RESULTS

Early season censuses in plots A and B revealed a differential effect of kangaroo paws density on the local abundances of male and female zaprochilines. Presumably owing to increased food levels, female abundance in plot A during the early season was approximately 50% higher than that recorded in plot B (Mann-Whitney test; U=58; $P < 0.01$; Table 1). In contrast, male abundance was, on average, not significantly different between plots A and B (Mann-Whitney test; U=22; $P > 0.05$; Table 1).

An intersexual difference in response to changing food levels was also apparent when seasonal trends in abundance were examined for plot A (Table 1). Female abundance varied significantly among monitoring periods (Kruskal-Wallis test; H=15.3; $P < 0.001$). Corresponding to an increase in flowering kangaroo paws, female numbers increased significantly between the early and middle monitoring periods (nonparametric multiple comparisons test; Zar, 1974; q=4.6; $P < 0.005$), whereas male abundance was similar between these 2 periods (q=1.8; $P > 0.05$). The number of flowering kangaroo paws declined dramatically by late season, and both male (q=4.2) and female (q=5.6) abundance decreased significantly from their mid-season levels (nonparametric multiple comparisons test; Zar, 1974; $P < 0.01$ in both tests).

Differences in male and female emigration rates for plots A and B were consistent with the inter-plot differences in abundance. In the early part of the kangaroo paws season, females emigrated from plot B at a greater rate than from plot A (Fig. 1; Table 2). For example, 36% of the females marked in plot A remained there for at least 9 days compared to only 9% of the females in plot B. Average residency of females was approximately 7 days for plot A but only 4 days for plot B. In contrast, no inter-plot difference in departure rates was noted for males (Fig. 1; Table 2), and approximately 15% of the males in both plots remained for at least 9 days. Average male residency

TABLE 1. ABUNDANCE OF MALE AND FEMALE *K. NARTEE* [MEAN (SD); N=8] AND FLOWERING KANGAROO PAWS (N=1) IN PLOTS A AND B.

	Males	Females	Kangaroo Paws
Plot A			
Early	27 (7)	25 (5)	146
Middle	25 (6)	31 (5)	307
Late	16 (7)	14 (5)	93
Plot B			
Early	25 (5)	16 (4)	50

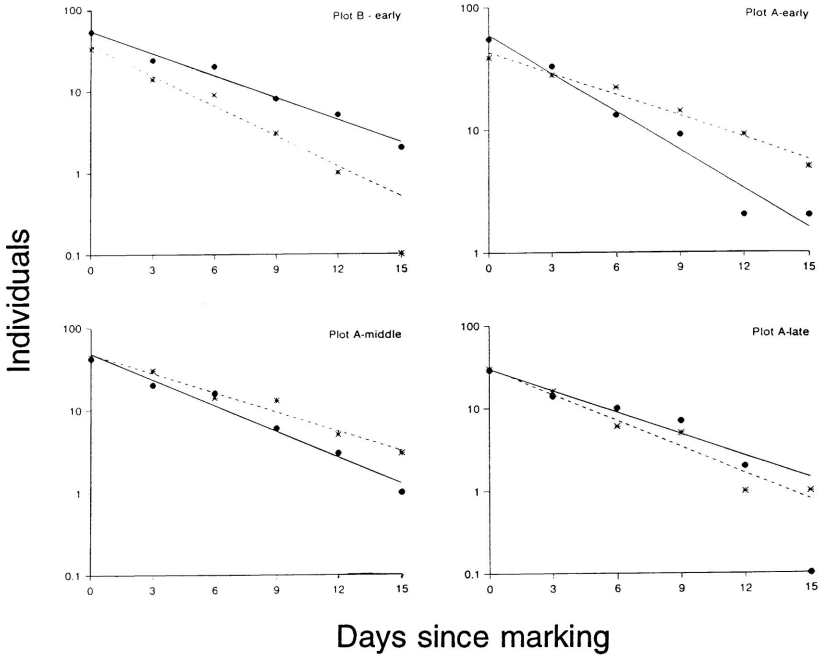

Fig. 1. Survivorship data for marked males (solid circles/lines) and females (open circles/dashed lines) in the two study plots. Numbers of individuals (log scale) remaining in the plot are graphed against days since marking. Linear regressions: Males (solid lines) - (a) Y=1.72-0.08X; r^2=0.93 (b) Y=1.73-0.09X; r^2=0.94 (c) Y=1.67-0.09X; r^2=0.98 (d) Y=1.54-0.09X; r^2=0.95. Females (dashed lines) - (a) Y=1.53-0.10X; r^2=0.98 (b) Y=1.63-0.055X; r^2=0.97; (c) Y=1.66-0.07X; r^2=0.97 (d) Y=1.45-0.08X; r^2=0.95.

was about 5 days in both plots A and B. As this result suggests, during the early season males left plot A at a greater rate than did females (Fig. 1; Table 2).

The degree to which emigration rates varied over the season also differed between the sexes. In plot A, female emigration rates showed a consistent increase through time: rates increased 30% between the early and middle period and 20% between the middle and late periods, though the latter difference was not statistically significant (Fig. 1; Table 2). Correspondingly, average female residency in plot A declined from 7 days in the early season to approximately 4 days by late season. In contrast, male emigration rates from plot A were similar between all monitoring periods (Fig. 1; Table 2). Owing to this difference, male and female emigration rates became more similar through time, with middle season values differing marginally and late season values not differing at all (Table 2).

Early season mapping of the positions of individual zaprochilines revealed that on most dates both males and females were randomly distributed in both Plots A and B. Males in plot A and females in plot B were randomly distributed on all 8 dates (P=.05 in these and all subsequent tests; t-test following Clark & Evans, 1954). Female distribution in plot A was random on 6 dates, aggregated on one date, and regular on one date. Male distribution on plot B was aggregated on one date and random on all others.

TABLE 2. MATRIX SUMMARIZING STATISTICAL COMPARISONS OF SLOPES OF THE SURVIVORSHIP CURVES (I.E., EMIGRATION RATES) SHOWN IN FIG. 1.

		Be[1]	Ae	Am	Al	
	Be	*	ns	ns	ns	
	Ae	***	**	ns	ns	Males
Females	Am	***	*	ms	ns	
	Al	ns	*	ns	ns	

[1]On the matrix borders, upper case letters (A,B) designate plot; lower case letters (e,m,l) designate early, middle, and late monitoring periods, respectively. Within the matrix, elements above the diagonal represent comparisons between male populations, and elements below the diagonal represent comparisons between female populations. Elements on the diagonal represent comparisons between male and female populations. ns - not significant; ms - marginally significant ($P < 0.1$); * - $P < 0.05$; ** - $P < 0.005$; *** - $P < 0.001$.

DISCUSSION

Consistent with our previous study (Shelly & Bailey 1992), male and female *K. nartee* exhibited different spatial distributions relative to food resources: in the early season comparison of the two plots, male abundance was similar between rich and poor food patches, whereas females were more numerous in the rich patch. Previously, we suggested that this difference might have reflected a sampling bias. Females were perhaps most conspicuous in dense patches of kangaroo paws, where they were often feeding on exposed flowers well above the ground. In contrast, males usually perched close to the ground regardless of the vegetation, and therefore our ability to locate males was, more or less, independent of the density of kangaroo paws. Although this possibility cannot be dismissed completely, the intersexual difference in movement described here is strong corroborative evidence that the intersexual difference in spacing was real and was not simply a sampling artifact.

It appears unlikely that predation (rather than emigration) accounted for the decay curves observed for marked individuals. If, as seems likely, predators (e.g., spiders, mantids) displayed an "aggregative response" (Hassell et al. 1976) to the zaprochilines (i.e., settled preferentially in areas of higher prey abundance), then we might have expected decay curves to be steeper in plot A (where zaprochiline abundance was higher) than in plot B. Yet, the opposite trend was observed for females, and decay curves for males were similar in the two areas. In addition, and independent of an aggregative response by predators, it remains difficult to explain why predation would have a differential sex-bias in the 2 study plots, i.e., why (in the early season) females would be more vulnerable to predation than males in plot B but less vulnerable in plot A?

The "uncoupled" spatial distributions of males and females observed during the kangaroo paws season contrasts dramatically with the situation described for the katydid *Orchelimum nigripes* Scudder (Feaver 1983). In this species, males initially established territories in areas of high female eclosion. However, females later moved to preferred oviposition sites, inducing males to relocate as well and establish new territories at the oviposition area. Moreover, males typically perched along the borders of the oviposition areas, where they had a higher chance of intercepting females. Based on these observations, Feaver (1983) concluded that males assessed territory locations, not on the basis of the resources present (otherwise males would have settled within oviposition areas independently of females), but by directly monitoring female abundance.

At present, it is not known what strategy male *K. nartee* use to maximize encounters with potential mates. Based on the present results, it appears unlikely that males use food resources or conspecific calling as a cue to female abundance (see also Bailey & Simmons 1991). It is possible that movement by females may have greatly reduced spatial variation in male mating opportunities. Despite their lowered emigration from rich food patches, females still displayed a relatively high level of movement: even when flowering kangaroo paws were abundant, 40%-50% of the females moved out of plot A within 3 days of being marked. Consequently, males in poor food patches may have encountered females travelling to new feeding or oviposition sites. If female traffic were sufficiently high over the entire habitat, the ability of males to differentiate areas of high and low female abundance may have been limited, leading to the even distribution of males over the habitat.

However, this explanation simply raises another unanswered question, i.e., why do female *K. nartee* move so frequently? The observation that female emigration from plot A increased between early and mid season (despite an increase in flowering kangaroo paws) suggests an age effect, perhaps related to increased searching for oviposition sites. Any tendency to disperse eggs, and thereby spread mortality risks, would further contribute to frequent movement by females. In short, it appears that understanding the movement and distributional patterns of females (and thus males) may require information on the oviposition behavior, particularly the identification of preferred egg-laying sites.

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TECHNIQUES FOR DIFFERENTIALLY STAINING *LIRIOMYZA TRIFOLII* (DIPTERA: AGROMYZIDAE) EGGS AND STIPPLES WITHIN COS LETTUCE LEAVES

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ABSTRACT

Different staining procedures were evaluated for their time requirements and effectiveness to differentially stain *Liriomyza trifolii* (Burgess)(Diptera: Agromyzidae) eggs and wounds (i.e., stipples) made by the female flies ovipositing within cos lettuce (*Lactuca sativa* L.) leaves. The best method for staining eggs within leaves was a lactophenol acid fuchsin solution that cleared the leaves of chlorophyll and stained the eggs pink to dark red. Modification of this established procedure reduced the total staining time to 7 min per leaf. Stipples were counterstained blue by immersing the leaves in a lactophenol cotton blue solution for 1 min immediately following the egg staining procedure. Larvae were not stained by either procedure. A new technique was devised for highlighting stipples using solutions of silver nitrate, sodium carbonate, formaldehyde and citric acid. The procedure resulted in blackened stipples that

were highly visible against the green leaves and did not kill developing larvae within the leaves. Eggs could later be stained using the lactophenol acid fuchsin technique. Sufficient contrast and color differences were produced by these procedures to successfully quantify stipples, eggs and larvae within stained and counterstained leaves from digitized microscopic video images using computer image analysis software.

Key Words: Serpentine leafminer, *Lactuca sativa*, lactophenol, acid fuchsin, cotton blue, silver nitrate.

RESUMEN

Fueron evaluados varios procedimientos para la tinción diferencial de huevos y heridas de *Liriomyza trifolii* (Burguess) producidos por la hembra en hojas de lechuga de la variedad Cos (*Lactuca sativa* L.). Los criterios evaluados fueron el tiempo que duró el procedimiento y la capacidad de teñir solamente los huevos y las heridas. Hasta ahora el mejor método para teñir los huevos dentro de las hojas es usar una solución de lactofenol y fuchsina ácida para aclarar la clorofila y teñir los huevos de rosado a rojo intenso. La modificación de este proceso establecido redujo el tiempo total de teñido a 7 min por hoja. Las punteaduras pueden ser contrateñidas mediante la inmersión de las hojas en una solución de lactofenol y azul de algodón por un minuto inmediatamente después del proceso de tinción del huevo. Las larvas no fueron teñidas mediante ningún proceso. Fué elaborada una nueva técnica para destacar las punteaduras usando soluciones de nitrato de plata, carbonato de sodio, formaldehído y ácido cítrico. El proceso dió como resultado punteaduras negras muy visibles contra el verde de las hojas, y no mató las larvas en desarrollo dentro de las hojas. Los huevos pueden luego ser teñidos usando la técnica del lactofenol con la fuchsina ácida. Hubo suficiente contraste y diferencias producidas por estos procedimientos para cuantificar exitosamente las punteaduras, huevos y larvas dentro de las hojas teñidas y contrateñidas a partir de imágenes de video usando software para análisis de imágenes de computadora.

Serpentine leafminers, *Liriomyza trifolii* (Burgess), are a major pest of lettuce (*Lactuca sativa* L.) and other crops, particularly in Florida (Genung & Janes 1975, Leibee 1981, Foster 1986). Damage to lettuce is in the form of stipples (i.e., scars from feeding and oviposition punctures), leafmines, and pupae within the lettuce heads. Hundreds of acres of lettuce are disked under annually before harvest, or left in the field during harvest because of leafminers. Costs to the industry, including lost production costs and pesticide applications, probably exceed $1 million annually in south Florida alone.

Host plant resistance is one method under examination to reduce leafminer pressure in lettuce. Field and laboratory studies indicated significant differences in stipple rates among commercial head and cos (i.e., romaine) lettuce cultivars (Nuessly & Nagata 1993, 1994). However, oviposition rates (based on counts of 2-d-old larvae within leaves) varied much less among romaine cultivars than was expected, based on overall stipple counts (unpublished data). This suggested that variation among cultivars mediated the feeding puncture to oviposition puncture ratio, or affected egg or early instar mortality. In order to identify the source of the observed variation, it was necessary to quantify both stipples and eggs.

Stipples on the distal areas of lettuce leaves can be observed using a 10X stereomicroscope with either direct or transmitted light. However, callous tissue and exudates that form over leaf punctures make it difficult to identify eggs, particularly in culti-

vars with thick leaves or with strong wound responses. Eggs and stipples in the proximal area of lettuce leaves are difficult to quantify accurately even under microscopic examination.

Many techniques are available for staining insects, nematodes, and fungal hyphae within plant tissue. However, these techniques may require several hours to days to complete the entire staining, destaining and counterstaining steps (e.g., Carlson & Hibbs 1962, Gilstrap & Oatman 1976, Simonet & Pienkowski 1977). Also, extensive variation in stippling and oviposition wounds within treatments on cos lettuce necessitates large sample sizes to determine meaningful differences among cultivars. Leaves are similar in size and shape, and stipple rates vary significantly with their location on the leaves and plants (Nuessly & Nagata 1994), thus test leaves need to remain separated to preserve their identity. This requires large holding spaces for test leaves if the staining procedures require hours or days to complete. Additionally, stipples on individual leaves or plants, which can number in the thousands, take considerable time to quantify. Magnified images of stipples on leaves acquired with a video camera and microscope, and then digitized into a computer, could be counted quickly if the stipples and eggs could be enumerated based on color. The purpose of this study was to compare several established staining techniques and to evaluate leaf-clearing and staining solutions and dyes, with the goal of finding techniques that would rapidly differentiate (< 1/2 h) stained eggs and stipples on cos lettuce.

Materials and Methods

Four cos lettuce cultivars were used in the evaluation: 'Floricos 83', 'Valmaine', 'Tall Guzmaine', and 'Paris Island Cos'. Plants were grown in a greenhouse using methods reported by Nuessly & Nagata (1994). The following methods were used for exposing plants to flies, handling after exposure and evaluating *L. trifolii* mortality and development in cos lettuce. Groups of four plants of the same cultivar were exposed to eight pairs of 48-h-old *L. trifolii* for 24 h within screened cages. After 72 h, all flies were removed, and then the plants were carefully re-caged for another 72 h to insure that no further oviposition occurred. Since larvae emerge from eggs within 70 h, any eggs detected in plants after this time could be assumed to be infertile. Time was allowed also for callous formation at the leaf punctures and for larvae to emerge from their eggs and start mining within the leaves. In addition, other leaves were processed within 24 h of oviposition to verify that the staining technique worked on both viable and inviable eggs. The leaves were excised near the leaf axil and processed individually. Stained leaves were examined with a stereomicroscope at 10 to 30 X using direct and transmitted light.

Most staining techniques for insects, nematodes, or fungal hyphae involve boiling the plant tissues in a solution to clear and stain them. This is followed by a destaining process that may require additional boiling. Tissue disruption can occur during this process. As a result, Parrella & Robb (1982) modified the lactophenol acid fuchsin procedure of Simonet & Pienkowski (1977) to reduce the boiling time. This reduced tissue disruption and improved staining of *L. trifolii* eggs in chrysanthemum, tomato and celery leaves. However, their technique still required the leaves to steep in the solution for ≥3 hr after boiling.

Beyond the time concerns, materials used in lactophenol staining procedures are relatively expensive when used in the volumes required for our studies. For example, the lactophenol acid fuchsin stock solution was made as follows: one part water; one part lactic acid; one part phenol; two parts glycerin; and 0.5 g acid fuchsin per 0.5 liter of solution. To address the cost concern, we evaluated other leaf-clearing and de-stain-

ing agents (acetic acid, ethanol, glycerin, lactic acid, phenol, polyethylene glycol and combinations) as well as insect and plant stains (cotton blue, methylene blue, acid fuchsin, methyl red, scarlet red, saffarin, and gentian violet) to find a technique suitable for our studies with lettuce. We also evaluated modifications of the following techniques developed for staining insect eggs and plant parasitic nematodes in plant tissue: lactophenol acid fuchsin (Parrella & Robb 1982), lactophenol cotton blue (Franklin & Goodey 1959), water methyl red (Curtis 1942), and ethanol scarlet red (Sugimoto 1976).

In addition, we evaluated a technique for highlighting stipples that involved excising leaves, washing them in distilled water to remove any surface contaminants, and then immersing them for 5 min in a 0.2% aqueous solution of silver nitrate. The leaves were then washed in distilled water and immersed in a 7% aqueous solution of sodium carbonate (with 0.375 ml 37% formaldehyde added per 100 ml solution) to develop the stain. After 3 min in the developer, the leaves were removed and immersed in a 20% aqueous solution of citric acid to stop the staining. A final distilled water wash completed the procedure. Care was taken not to handle the leaves without gloves, or to abrade or tear the leaves prior to staining, as these areas would also become stained and mask the stipples.

Results and Discussion

We found that the shape of the container used for the staining procedure ultimately affected the quality of the stain. For example, bending or folding the leaves into beakers resulted in tissue destruction and too much dye infusion. Thus, to maintain the structural integrity of the leaves, it was necessary to use a container large enough for the entire leaf to lay horizontally in the solution. We found that a glass loaf dish, 24 × 14 × 7.5 cm (L × W × D), was large enough for the cos lettuce leaves, and small enough for the entire bottom surface of the dish to remain in contact with the heating surface of a hot plate. One-half liter of stain solution provided adequate volume for immersing the leaves during staining without touching the bottom of the dish. The leaves were held immersed approximately 1.0 cm below the surface of the test solutions with a strainer made from 5 mm diam glass rods.

Our goal was to develop procedures by which all staining, destaining and counterstaining could be completed in 15 min. We found it was necessary to boil the leaves in order to achieve leaf-clearing and egg-staining within this time limit, even though this might result in some tissue disruption. Without boiling, the eggs were not sufficiently stained to discern them from the background color of the leaves.

Our preliminary tests with cos lettuce leaves indicated that boiling moderate to older aged leaves >1 min, or young leaves >30 s, caused partial separation and tearing of the abaxial and adaxial epidermal surfaces. This resulted in either excessive or limited staining of the compromised tissue, depending on the staining solution.

Leaves boiled in 50, 60, and 70% ethanol produced irregular clearing and the solution evaporated quickly. A boiling aqueous solution of 10% acetic acid and 50% ethanol cleared the leaves well, but again this solution evaporated quickly and was potentially dangerous because of alcohol's' flammability. Adding stains to the acetic acid-ethanol solution produced various results. Methylene blue stained the leaf tissues blue, but not the stipples or eggs. Methyl red heavily stained the leaf tissues and stipples, but not the eggs. There was not enough contrast between eggs and stained leaves to be able to easily identify the eggs. Gentian violet and saffarin poorly stained the leaf tissues, stipples and eggs.

The egg staining methods of Curtis (1942) and Sugimoto (1976), who used final staining solutions of water saturated with methyl red and 70% ethanol saturated

with scarlet red, respectively, did not stain *L. trifolii* eggs in lettuce. Lettuce leaves were nearly destroyed by the three separate boiling steps used by Curtis (1942). Other published methods of insect staining were not tested because they involved two separate boiling procedures or long (i.e., > 24 h) staining times.

The lactophenol acid fuchsin solution of Simonet & Pienkowski (1977) provided the best staining of *L. trifolii* eggs and the best overall clearing of leaves. Liquid phenol produced the most consistent results and was easier to work with than phenol crystals. Modifications of their technique allowed us to successfully prepare the relatively soft lettuce tissue. The leaves were cleared and stained in a slow boiling solution to minimize tissue disruption. Total staining time was ultimately reduced to 7 min per leaf.

The exact procedure we used for staining eggs in lettuce leaves was as follows: leaves were placed in the boiling solution and held immersed for 1 min; young leaves with soft tissue required boiling for only 30 s; the solution was removed from the hot plate and the leaves allowed to steep in the stain for an additional 3 min; leaves were removed from the stain and rinsed in warm water for 3 min to remove excess acid fuchsin; stained leaves were then placed in cold water within 15 cm diam glass petri dishes for microscopic examination.

This modified procedure stained the eggs pink to deep red and made them easy to locate within the leaves. The 4 min staining and clearing procedure did not completely clear the leaves of chlorophyll, but the degree of clearing was sufficient to make it easy to locate the eggs. Leaves cleared better if they were boiled >1 min or steeped >3.5 min, but this resulted in greater destruction and darker staining of leaf tissues, respectively. Both of these conditions proved unsuitable for our purposes.

Efforts to remove or replace phenol from this procedure were unproductive. When phenol was left out and the volume of water in the solution was doubled, or when phenol and water were replaced with an equal volume of polyethylene glycol, the leaves became stained too heavily to be de-stained within several hours and the eggs were poorly stained. The addition of 0.1 part acetic acid to either of these solutions without phenol improved leaf-clearing and egg staining, but the eggs were not stained as well as when phenol was in the solution.

While this rapid lactophenol acid fuchsin technique produced well-stained eggs, it did not stain all of the stipples. Many became indistinguishable once the leaves were cleared. Parrella & Robb (1982) found that longer staining periods (≥ 3 h) with acid fuchsin stained stipples pink to red on chrysanthemum, celery, and tomato leaves. However, lettuce leaves steeped 10 min in lactophenol acid fuchsin became darkly stained, while the stipples still could not be differentiated from normal leaf tissue. Increasing steeping times made the eggs difficult to locate, so an alternative to longer staining with acid fuchsin was desired. It was determined that stipples could be quickly counterstained using a lactophenol cotton blue solution (Franklin & Goodey 1959) immediately following the egg staining procedure. The counterstain solution was identical to the lactophenol egg staining solution except the acid fuchsin was replaced with 0.4 g cotton blue per 0.5 liter of solution. The solution was initially boiled and then allowed to cool to room temperature (25 ± 1°C) before use. Following the 3 min water rinse after the lactophenol acid fuchsin egg stain, leaves were placed in the lactophenol cotton blue solution for 1 min. Leaves were then washed in warm water for 1 min to remove excess stain. This procedure successfully stained the stipples blue which provided a good contrast against the pink leaf tissue. Leaves left in the cotton blue solution for >1 min absorbed excessive stain that blurred the boundaries of individual stipples. Although stipples could still be manually counted, the loss of independent boundaries around each stipple compromised efforts to use computer assisted

image analysis for stipple counts. Efforts to combine the egg and stipple staining steps failed. Leaves boiled in lactophenol acid fuchsin-cotton blue solution resulted in the eggs being stained purple, while the stipples were unstained.

Larvae were not stained by acid fuchsin or cotton blue in lactophenol. However, transmitted light made them appear yellow to greenish brown against the pink background of the leaves following the egg and stipple staining steps. Tunnels within the leaves were occasionally stained pink and blue.

While the lactophenol-cotton blue treatment was satisfactory for intact leaves, it poorly stained stipples in areas of tissue disruption and in areas closely adjoining primary leaf veins at the proximal region of leaves. Thus, the best overall technique for highlighting stipples turned out to be the silver nitrate method. Black silver particles adhered to all damaged portions of leaves and revealed all stipples, even if there was no visible wound response. The blackened stipples provided enough contrast against the green leaves for them to be successfully counted with computer image analysis software. Using this technique offers several benefits. None of the solutions used in this procedure masked or removed markings made on the leaves with felt tipped indelible ink pens, so several leaves could be processed in the same container without loss of leaf identity. These chemicals did not adversely alter leaf or egg tissues, so they could be later stained using the lactophenol acid fuchsin solution to locate eggs. Since there was no boiling involved, the procedure also allowed larvae within the leaves to successfully complete development.

There was no obvious difference in leaf-clearing or egg and stipple staining among the four cos lettuce cultivars examined. Inviable eggs were stained the same color by lactophenol acid fuchsin as viable eggs. Chorion of empty eggs absorbed little of the acid fuchsin stain.

In spite of the costs of the lactophenol procedures, they worked the best with the dyes to clear leaves and stain target tissues. The modified lactophenol acid fuchsin, lactophenol cotton blue, and silver staining procedures all met our objectives of rapid techniques that would differentially stain eggs and stipples. They produced adequate contrast for counting eggs, stipples, and larvae in one step, and proved suitable for use with computer image analysis software, e.g., Optimas (Optimas Corp., Edmonds, Washington). They will be used to further our understanding of mechanisms involved with host plant resistance to serpentine leafminer in lettuce.

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COMMUNITY STRUCTURE OF HOUSE-INFESTING ANTS (HYMENOPTERA: FORMICIDAE) IN SOUTHERN BAHIA, BRAZIL

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ABSTRACT

The community structure of ants which infest houses in southern Bahia was studied. Of the 31 species collected, 8 are considered as characteristic of human habitat, even though some of them are also found in other conditions, such as in regional agrosystems. *Pheidole megacephala* was the dominant species and showed a negative association with most other ants, while *Tapinoma melanocephalum* was almost always found with other ant species. The notable absence of the species *L. humile* in our tests may be due to the antagonistic effects of *Ph. megacephala*.

Key Words: *Tapinoma melanocephalum*, *Pheidole megacephala*, *Linepithema humile*, Bahia, Brazil.

RESUMÉ

La structure de la communauté de fourmis qui infestent les habitations dans le sud de Bahia a été étudiée. Parmi les 31 espèces rencontrées, 8 sont considerées caractéristiques de l'habitat humain, bien qu'elles soient aussi rencontrées dans d'autres conditions, telles que dans certains agrosystèmes régionaux. Dans les communautés, *Pheidole megacephala* est dominante et montre une association négative avec la plupart des autres fourmis, alors que *Tapinoma melanocephalum* est à peu près toujours rencontrée avec d'autres espèces. L'absence notable de *Linepithema humile* de nos tests est probablement liée à son antagonisme avec *Ph. megacephala*.

Ants which infest houses have only recently received attention from entomologists in South America (Brown 1964, Fowler et al. 1992, 1993, Ketelhut et al. 1993; Bueno & Fowler 1994) in contrast to the Northern hemisphere, where ants which occur in houses and hospitals have been studied for at least two or three decades (see the reviews of Smith 1965, Edwards 1986, Eichler 1990, Thompson 1990). Compared to temperate environments, urban tropical environments may yield ideal conditions for a range of species for foraging and nesting. However, in both the temperate and tropical urban conditions of Americas, ants which occur in the human environment are regarded as destructive or detrimental to human health, food conservation and quality, wood conservation, electrical installations, and electronic equipment (Smith 1965, Fowler 1990, Thompson 1990, Vinson & McKay 1990, Fowler et al. 1993a & b).

A higher frequency of occurrence of ants in houses in tropical regions of South America compared to houses in temperate regions (Fowler et al. 1993, Bueno & Fowler 1994) is expected because of the favorable climatic conditions and the characteristics of human habitation in the tropics. To understand the community structure of house-infesting ants in tropical regions, a cooperative study was conducted in the State of Bahia, Brazil, by the Universidade Estadual de Santa Cruz (UESC) and the Myrmecology Laboratory of the Cocoa Research Center (CEPEC), at Ilheus, with the aim of describing the ant situation in houses of this region. This study is the first step toward a larger study on the interactions between ants and human health in tropical environments.

MATERIAL AND METHODS

One hundred houses were randomly sampled for ants in the region of Ilheus (14°45'S, 39°13'W), in the southern portion of the State of Bahia. Ants were caught in small glass test-tubes (length: 50 mm; diam: 7 mm) using honey as bait. In each house, three test-tubes were placed on the floor in each room: livingroom, bedroom, kitchen, and bathroom. Tubes were collected in the morning, 8 to 12 hours after initial placement. Collected test-tubes were transported to the Myrmecology Laboratory of the CEPEC where the ants were identified to species.

Data were analysed using a house or a room as the study unit. An evaluation of interrelations between the most common house-infesting ant species and their possible associations with other species were tested by chi-square analysis with Yates' correction applied (Siegel 1956). This procedure has previously been used by Room (1971) and Majer et al. (1994) to study the ant mosaic in cocoa plantations.

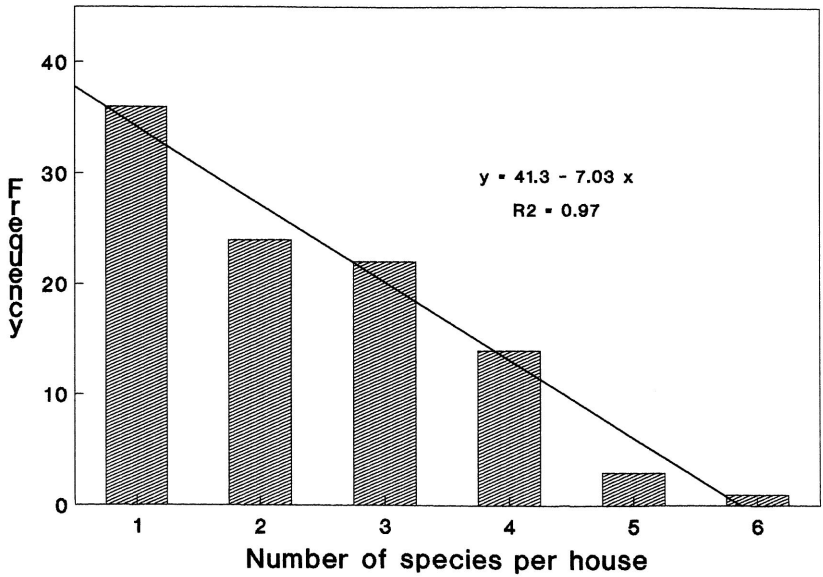

Fig. 1. Frequency of houses infested by ants as a function of the number of species found per house.

Results and Discussion

All houses sampled were occupied by at least one species of ant. A total of 31 species belonging to 14 genera and 4 subfamilies were found: Ponerinae: *Gnamptogenys* [1 sp.], *Odontomachus* [1 sp.]; Myrmicinae: *Acromyrmex* [1 sp.], *Crematogaster* [1 sp.], *Monomorium* [2 spp.], *Pheidole* [5 spp.], *Solenopsis* [4 spp.], *Tetramorium* [3 spp.], *Wasmannia* [1 sp.]; Dolichoderinae: *Dorymyrmex* [1 sp.], *Tapinoma* [1 sp.] and Formicinae: *Brachymyrmex* [1 sp.], *Camponotus* [7 spp.]), *Paratrechina* [2 spp.]. Eight exotic species (25.8%), *Monomorium floricola* (Jerdon), *M. pharaonis* (L.), *Paratrechina longicornis* (Latr.), *Pheidole megacephala* (Fabr.), *Tapinoma melanocephalum* (Fabr.), *Tetramorium bicarinatum* (Nyl.), *T. lucayanum* Wheeler and *T. simillimum* (Fr. Smith) (Delabie 1993) were responsible for 68.9% of the total number of occurrences. Most of these ants are known as "tramp ants" and have characteristics in common, such as polygyny, low intraspecific aggressivity, and the ability to change their nest site easily (Passera 1993).

One to six species were found per house, and the frequency observed for each of these six classes decreased inversely to the number of ants found (Fig. 1). The greatest number of ant occurrences (29%) and species (22) was observed in the livingroom (Fig. 2A,B). In Bahia, the livingroom is generally the largest room in the house and probably also offers more possibilities for nest sites (e.g., plant containers) to a variety of species. The other three rooms sampled (bathroom, bedroom and kitchen) showed a similar range of species number (14-16) and ant species occurrence (22-27%) (Fig. 2A,B). The kitchen was the location with the highest number of species and occurrences and the bedroom the lowest.

Among the 31 species collected, eight (Table 1) could be considered to be truly characteristic of human habitats because they were found at different sites and in all parts

TABLE 1. RELATIVE FREQUENCY OF MOST COMMON SPECIES OF HOUSE-INFESTING ANTS IN SOUTHERN BAHIA (>10 OCCURRENCES AND LIVING EVERYWHERE IN HOUSES).

Ant species	% of Houses	% of Occurrences
Pheidole megacephala	47.0	31.7
Tapinoma melanocephalum	48.0	22.9
Solenopsis saevissima	23.0	9.3
Paratrechina longicornis	18.0	7.9
Wasmannia auropunctata	12.0	5.5
Camponotus (Tanaemyrmex) sp	13.0	4.8
Pheidole sp4	13.0	3.8
Tetramorium simillimum	9.0	2.9

of the house. These eight species were responsible for 88.9% of the total number of occurrences. In Bahia, these ants are not found exclusively in houses, but are also commonly found in gardens, secondary vegetation, and crops. Their distribution tends to be different at higher latitudes where few species are limited exclusively to human dwellings, e.g., *M. pharaonis* (Eichler, 1990). This difference is probably related to the year-round thermal stability in this region, which allows these ants to live both in and out of human habitats.

The most abundant ant species were *Ph. megacephala* (47% of infested houses and 31.7% of occurrences) and *T. melanocephalum* (48% of infested houses and 22.9% of occurrences).

The community structure at the room level (Fig. 3) showed that *Ph. megacephala* was the only species that was truly dominant (see Majer et al. 1994). This ant was negatively associated with the species *P. longicornis*, *Solenopsis saevissima* (Fab.), *T. melanocephalum* and the little fire ant *Wasmannia auropunctata* (Roger). *Ph. megacephala* was frequently the only species in a room or a house, with all potential competitors being excluded (Fig. 4). In contrast, the second most common species, *T. melanocephalum*, usually occurred with other ants (Fig. 4). However, it seems that

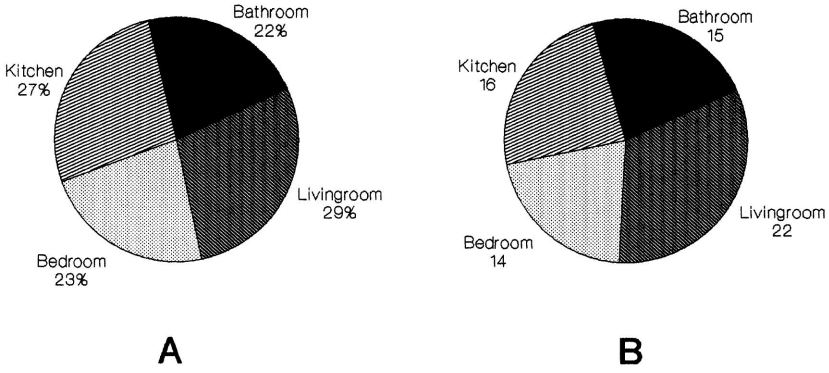

Fig. 2. Percent of ant occurrences (A) and number of species (B) in sampled rooms.

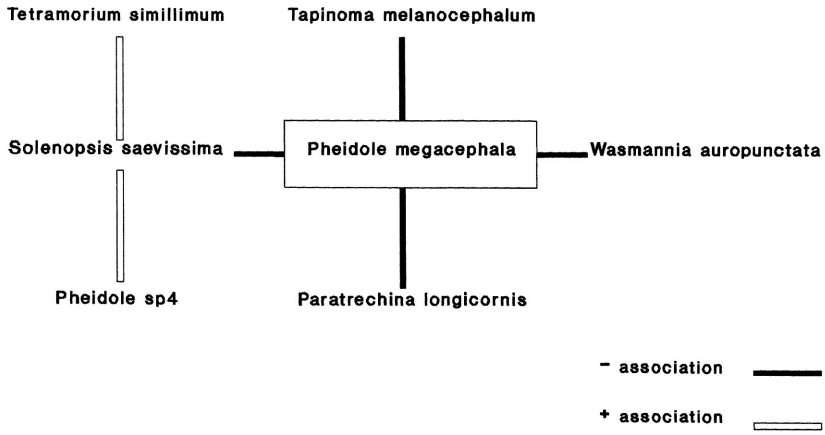

Fig. 3. Community structure of house-infesting ants of Bahia.

this species can act as a rapid colonizer, because it was the only species found when houses were new.

The study of size classes shows a bimodal distribution (Fig. 5) in which the smaller ants (< 5mm) were more common. Most of the larger ants were species of the *Camponotus* genus which are generally more active at night in Bahia (see, for example, Delabie et al. 1991). Establishment of these large ant species in the house environment may relate to their nocturnal behavior which does not interfere with the human activity and removes them from competition with diurnal species.

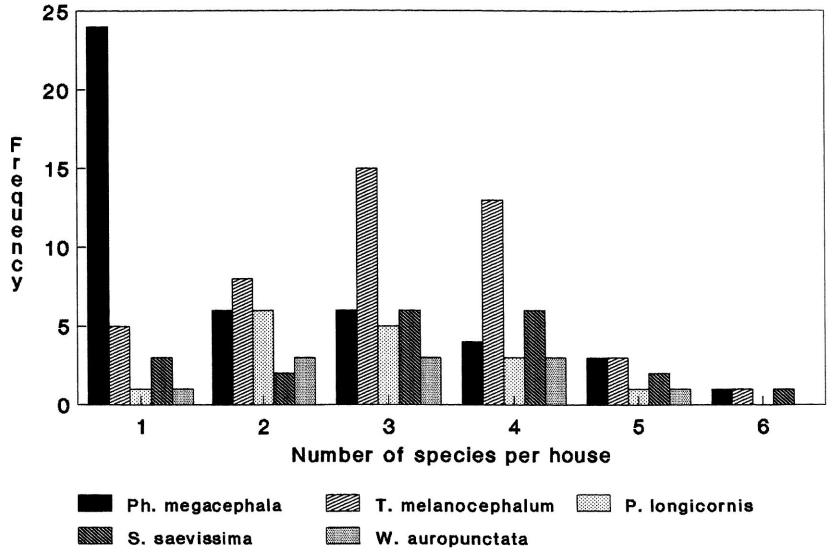

Fig. 4. Relative abundance of the 5 commonest ant species in Bahia houses.

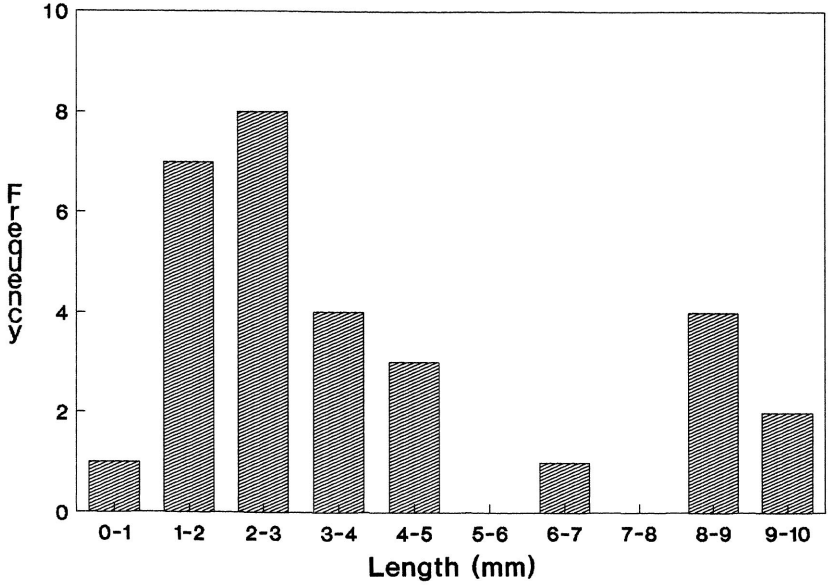

Fig. 5. Distribution of house-infesting ants as a function of body length.

Linepithema humile (Mayr) was completely absent from our house samples. This absence has been confirmed in all of south Bahia and confirmed by other extensive experiments over the region (J. H. C. D., unpublished data). However, the same species was commonly found forming discrete societies in cocoa plantations within the same region. It seems that *L. humile* avoids human settings in Bahia and this may be due to an antagonism with *Ph. megacephala* which is dominant in houses and other strongly human anthropized habitats. The antagonism between these two species has been documented in other situations (Haskins & Haskins 1965, Crowell 1968). Thus, the main difference between house-infesting ant communities in Bahia and other parts of Brazil is that *L. humile* can be found everywhere in the urban environment (Brown 1964, Fowler et al. 1993), while it seems restricted to only a few agricultural situations in Bahia.

Acknowledgments

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EFFECTS OF FOREST MANAGEMENT PRACTICES ON TERRESTRIAL COLEOPTERAN ASSEMBLAGES IN SAND PINE SCRUB

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ABSTRACT

Coleopteran assemblages were sampled monthly for one year using pitfall traps in replicated sites of three 5- to 7-year-old disturbance treatments and mature forested sand pine scrub in the Ocala National Forest, Marion County, Florida. Disturbance treatments were (1) burning at high-intensity and salvage-logging; (2) clearcutting, roller-chopping and broadcast seeding, and; (3) clearcutting and bracke-seeding. Community similarity of coleopterans was high. No differences in species richness, diversity, density, or evenness were detected. Of 40 species captured, only seven were common (n > 50). Predaceous beetles were numerically dominant followed by scavengers. Few xylophagous or herbivorous coleopterans were captured, probably due to trap bias. Peaks of annual above-ground terrestrial activity varied among species. An absence of differences among treatments may reflect similar plant communities or structural habitat features. Additionally, a dearth of mature forest specialists might be predicted in systems where mature forest was historically rare due to large-scale, high-intensity, and low-frequency wildfire.

Key Words: Beetle assemblage, clearcutting, wildfire.

RESUMEN

Durante un año fueron muestreadas mensualmente comunidades de coleópteros usando trampas de suelo en sitios replicados que poseen tres tipos de tratamientos con 5 a 7 años de edad, y el matorral que crece bajo un bosque maduro de pino de arena (sand pine) en el Bosque Nacional de Ocala, en el condado de Marion, en la Florida. Los tratamientos fueron (1) quema de gran intensidad y preservación de los troncos; (2) tala, corte de los troncos en pedazos y siembra de semillas al voleo, y (3) tala y siembra de semillas en los claros talados. La similaridad de comunidades de coleópteros fué elevada. No se encontraron diferencias en riqueza de especies, diversidad, o densidad. De 40 especies capturadas solamente siete fueron comunes (n > 50). Los escarabajos depredadores fueron numéricamente dominantes, seguidos por los comedores de carroña. Fueron capturados pocos coleópteros xilófagos o herbívoros, probabemente debido a los tipos de trampas. Los picos de actvidad terrestre por encima del suelo variaron entre las especies. La falta de diferencia entre los tratamientos puede reflejar comunidades de plantas o formas de habitats estructurales similares. Adicionalmente, la falta de especialistas de bosque maduro podría ser pre-

dicha en sistemas donde el bosque maduro es históricamente raro debido al fuego a gran escala, alta intensidad y baja frecuencia.

Coleopteran assemblages often are useful indicators for monitoring effects of land management practices on biodiversity (Eyre et al. 1989; Kremen et al. 1993). Many species have specific habitat requirements and are capable of dispersing as habitat suitability declines (Refseth 1980). Also, the microcosm of food web dynamics, niches, and habitat requirements represented within the order Coleoptera permits insight into ecological shifts as land use changes.

Historical changes in climate and sea level, and subsequent isolation have led to a high endemism of plants (Christman & Judd 1990), vertebrates (Auffenberg 1982), and invertebrates (Deyrup 1989) in scrub. Knowledge of scrub arthropod distribution sheds light on the biogeography of Florida scrub but is poorly documented (Deyrup 1989).

The scrub ecosystem occurs in xeric, infertile sandy soils (Kalisz & Stone 1984) along coastal areas and inland ridges of Florida and extreme southern Alabama. The thick, sclerophyllous shrub layer is dominated by myrtle oak (*Quercus myrtifolia* Willd.), sand live oak (*Q. geminata* Small), Chapman's oak (*Q. chapmanii* Sarg.), rusty lyonia (*Lyonia ferruginea* Nutt.), and two species of palmetto (*Serenoa repens* Small and *Sabal etonia* Swingle ex Nash.). Herbaceous groundcover is scant. Sand pine (*Pinus clausa* Vasey ex Sarg.) scrub is a habitat variant containing sand pine as a dominant component.

The peninsular Florida variety of sand pine, *Pinus c. clausa*, has serotinous cones. Here, the naturally even-aged, monospecific sand pine canopy is maintained by the release of copious quantities of seed (recorded as > 2.47 million per ha, Cooper et al. 1959) following stand-replacing wildfire. Historically, low-frequency, high-intensity, and large-scale wildfire created a forest mosaic of temporally shifting age-classes (Rawlings 1933, Webber 1935, Bartram 1955, Myers 1990). An open, shrub-dominated habitat was maintained between wildfires, while mature forest probably existed intermittently in time and space, especially in sites protected from fire. The largest remaining area of scrub occurs in the Ocala National Forest in central Florida. Current forest management of sand pine scrub there entails clearcutting patches of approximately 8-24 ha. Heavy machinery used during the clearcutting operation crushes and kills nearly all above-ground vegetation. Clearcutting is commonly followed either by roller-chopping and broadcast seeding or "bracke-seeding." Roller chopper blades penetrate the soil to a maximum depth of 15 cm. Soil surface disturbance with this method is nearly complete. Bracke-seeding entails direct seeding along small, machine-created ridges (about 8 cm high). This method patch-scarifies approximately 30% of the soil surface (Outcalt 1990). Because of the wood fiber value and the possibility of large-scale, uncontrolled burns, fires in sand pine stands are usually extinguished as rapidly as possible. Normally, burned sites are salvage-logged.

Plant community recovery and habitat structure of clearcuts are similar in many respects to community recovery and structure following high-intensity wildfire (Campbell & Christman 1982, Abrahamson 1984a, 1984b, Schmalzer & Hinkle 1992, Greenberg et al. 1995). Major differences include (1) the absence of fire-associated cues for attracting pyrophyllic coleopterans to clearcuts; (2) the presence of few standing trees or snags in clearcuts versus an abundance of snags for several years follow-

ing a wildfire (unless salvage-logged, as in this study); (3) more slash piles and less bole-sized woody debris in clearcuts (personal observation), and; (4) landscape patterns such as patch size and connectivity.

Because it occurs on ideal sites for citrus and urban development, sand pine scrub is fast becoming and endangered ecosystem (Myers 1990). It is critical that the scrub ecosystem on public lands be managed to maintain the characteristic species diversity associated with the ecosystem and its driving processes.

This study is one portion of a larger study comparing plant, bird, and herpetofaunal communities among four stand treatments: intense burning and salvage-logging; clearcutting followed by either roller-chopping or bracke-seeding; and naturally regenerated, mature sand pine scrub. Here we describe and quantitatively compare coleopteran assemblages among these treatments. The study also provides information on annual cycles of commonly captured coleopterans.

Materials and Methods

Coleopterans were sampled using drift fences and pitfall traps in three replicated 5- to 7-year-old disturbance treatments and mature forested sand pine scrub (n = three sites each) in the Ocala National Forest, Marion County, Florida (Table 1). Disturbance treatments were (1) high-intensity burning, salvage-logging, and natural regeneration (HIBS); (2) clearcutting, roller-chopping, and broadcast seeding (RC), and; (3) clearcutting and bracke-seeding (BK). Mature (≥ 55 yr) sand pine stands that had naturally regenerated following a stand-replacing fire in 1935 were used as a control (MF). All sites had similar elevation, topographic, and soil characteristics; same pretreatment age, and (known) disturbance history (identical to MF); same post-treatment age for HIBS, RC, and BK sites (within 1.5 yr) (Table 1); were greater than 8.5 ha; and were more than 0.9 km from known water sources.

Sand pine density and height were measured in five 100-m^2 plots per site (or in a 20 m^2 subplot if density was high). Three 10-m line transects were randomly established within quadrats to quantify percent cover of the vegetation and microsite characteristics by category, including herb, shrub, pine, woody debris, leaf litter, and bare ground, using the line intercept technique (Mueller-Dombois & Ellenberg 1974). In MF, sand pine canopy cover was estimated using a spherical densiometer at the midpoint of each line transect.

Trapping arrays were designed and concurrently used for herpetofaunal sampling (Greenberg et al. 1994) but proved effective in sampling surface-active terrestrial arthropods as well. Arrays (modified from Campbell & Christman 1982) consisted of eight 7.6-m lengths of erect 0.5-m-high galvanized metal flashing arranged in an "L" shaped pattern with a 7.6-m space between each length. Two black 18.9-liter plastic paint buckets (pitfall traps) with 28.5-cm diam were sunk flush with the ground at both ends of each fence (n = 16 pitfall traps per site). To improve drainage, 1.25-cm holes were drilled into the bottoms of the pitfall traps. Drill holes were blocked with sticks to prevent escape. No killing agents were used in pitfalls. Arrays were located a minimum of 25 m from roads or stand edges (except for two drift fences of one array). We assumed that consumption of arthropods by vertebrates was minimal due to low capture rates of vertebrates during arthropod sampling periods. Any effects were consistent among treatments.

Arthropods were trapped for one 48-hour period each month from October 1991 through September 1992. Coleopterans were preserved in ethyl alcohol.

One-way analysis of variance (ANOVA) (SAS 1989) was used to determine differences among treatments in total numbers of individuals trapped, total numbers by

TABLE 1. DATES OF TREATMENT ADMINISTRATION AND VEGETATION SAMPLING IN THREE TREATMENTS AND MATURE FORESTED SAND PINE SCRUB.

Treatment	Burn	Clearcut or Salvage	Site Preparation	Sand Pine Seed	Sample
Burn-salvage (HIBS)	May 1985	June-Oct 1985	N/A	N/A	Summer 1991
Chop (RC)	N/A	Apr '83-Feb '85	June '86	Winter '86-'87	Summer 1991
Bracke (BK)	N/A	Fall '86	Winter '86-'87	Winter '86-'87	Summer 1991
Mature (MF)	Spring 1935	N/A	N/A	N/A	Summer 1991

species, Shannon's diversity indices, species richness, and evenness (Brower & Zar 1977). Horn's Index of Community Similarity (Horn 1966) was used to compare community overlap among treatments.

Each species was assigned to one of four feeding guilds: predator, scavenger (carrion and dung beetles), herbivore (root-, stem-, foliage-, or flower-feeding beetles), and xylophage based on adult food habits. ANOVA was used to detect differences in coleopteran density for each feeding guild.

Annual above-ground terrestrial activity cycles were estimated from captures of each commonly trapped species ($n \geq 50$ individuals caught over the 1-year period) for each trapping period.

Results

Mature forest differed structurally from disturbance treatments in having lower stem density and greater foliar cover and height of sand pine. Mature stands also had less bare ground and higher leaf litter, nonwoody plants (primarily lichens), and shrub cover than disturbance treatments (Table 2) (see also Greenberg et al. 1995).

A total of 1,849 beetles representing 40 species in 14 families was captured (Table 3). Only seven species were commonly captured (≥ 50 individuals). Two carabid species of *Pasimachus* (*P. strenuus* LeConte and *P. subsulcatus* Say) were dominant followed by the tenebrionid *Polopinus youngi* Kritsky and an undescribed tenebrionid species of *Helops*.

Three females of *Romulus globosus* Knull (Cerambycidae), a rare scrub endemic, were captured in the July trapping period. Based on collection dates of other specimens, this species is most active in June and July (Thomas 1991). *Peltotrupes youngi* Howden, a species endemic to the Ocala National Forest scrub in Marion and Putnam counties (Woodruff 1973), was relatively abundant. There were no differences in density of individual species among treatments (Table 3).

There were no differences among treatments in density, diversity, or evenness of captured coleopterans (Table 4). Species richness was lower in MF than in any of the disturbance treatments, but differences were not statistically significant (Table 4). Horn's Index of Community Similarity indicated a high degree of community overlap among all treatments (Table 5).

Feeding guild structure did not differ among treatments (Table 3; Fig. 1). Predaceous beetles were dominant, composing 63-73% of total beetle numbers, followed by scavengers (21-30%), herbivores (5-9%) and xylophages (≤ 1%).

Two general patterns of annual above-ground terrestrial activity cycles were apparent. Among completely trapped species, *Peltotrupes youngi* Howden and *Helops* sp. appeared to be most active above-ground from December-March and inactive mostly from June-October. Several other species were active above-ground from at least May-October (*Polypleurus* sp. from April-August) but inactive during the cooler winter months (Fig. 2).

Discussion

Several studies report decreases in forest specialists but increases in overall coleopteran diversity following forest disturbance, fragmentation, or deforestation as species of open habitat invade and mature forest generalists persist (Lenski 1982, Baguette & Gerard 1993, Buse & Good 1993, Halme & Niemela 1993, Niemela et al. 1993). Conversely, Niemela et al. (1988) found few differences in carabid assemblages

TABLE 2. STRUCTURAL CHARACTERISTICS (MEAN ± SE) OF SAND PINE SCRUB HABITAT IN THREE 5-7 YEAR POST-DISTURBANCE TREATMENTS AND 55-YEAR-OLD MATURE FOREST (N = FIVE SITES PER TREATMENT), OCALA NATIONAL FOREST, FLORIDA. DIFFERENT LETTERS DENOTE SIGNIFICANT DIFFERENCES AMONG TREATMENTS ($P < 0.05$).

	Pine			Shrub	Non-woody Plants	Leaf Litter	Bare Ground	Woody Debris
Treatment	Stems/ha	Height (m)	Foliar (% Cover)	% Cover				
Burn-salvage (HIBS)	4076.0[a]	2.7[a]	41.8[a,b]	51.2[a,b]	6.8[a,b]	66.6[a,b]	16.8[a]	20.3[a]
	653.0	0.1	11.7	7.9	3.4	7.3	4.9	8.1
Chop (RC)	3496.0[a]	2.8[a]	45.8[a]	42.9[a]	13.7[a]	70.2[a]	22.9[a]	2.1[b]
	270.1	0.1	9.0	7.3	6.4	7.1	5.9	2.2
Bracke (BK)	3080.0[a]	1.9[b]	21.2[b]	59.0[b]	4.9[b]	57.1[b]	21.5[a]	20.8[a]
	388.4	0.1	7.5	8.1	2.1	8.6	6.5	6.7
Mature (MF)	641.7[b]	16.7[c]	83.9[c]	73.4[c]	35.9[c]	99.6[c]	0.3[b]	5.3[c]
	64.8	0.6	4.5	7.8	10.3	0.6	0.5	1.8

TABLE 3. TOTAL NUMBER OF COLEOPTERANS CAPTURED IN EACH OF THREE TREATMENTS AND MATURE FORESTED SAND PINE SCRUB FROM OCTOBER 1991 THROUGH SEPTEMBER 1992 IN THE OCALA NATIONAL FOREST, MARION COUNTY, FLORIDA.

Guild[1]	Species	Treatment: Burn-salvage (HIBS) (n = 3)	Chop (RC) (n = 3)	Bracke (BK) (n = 3)	Mature (MF) (n = 3)
	ALLECULIDAE	—	—	1	—
H	*Hymenorus* sp.	—	—	1	—
	BURPRESTIDAE	3	2	2	
X	*Chalcophora virginiensis* Drury	3	2	2	—
	CARABIDAE	299	251	365	292
P	*Apenes opaca* LeConte	—	—	1	—
P	*Apenes sinuata* (Say)	1	—	—	—
P	*Carabid* sp. 1	—	1	—	—
P	*Carabid* sp. 2	—	1	—	—
P	*Cyclotrachelus faber* (Germar)	4	—	1	3
P	*C. hernandensis* Van Dyke	—	—	1	—
P	*C. morio* (Dejean)	—	—	1	—
P	*C. ovalum* Chaudoir	3	12	1	1
P	*Harpalus caliginosus* Fabricius	—	—	1	—
P	*Helluomorphoides clarvilleii* (Dejean)	3	14	16	8
P	*Pasimachus strenuus* LeConte	199	163	207	166
P	*P. subsulcatus* Say	89	60	136	114

[1]H = Herbivore; X = Xylophage; P = Predator; S = Scavenger.

TABLE 3. (CONTINUED) TOTAL NUMBER OF COLEOPTERANS CAPTURED IN EACH OF THREE TREATMENTS AND MATURE FORESTED SAND PINE SCRUB FROM OCTOBER 1991 THROUGH SEPTEMBER 1992 IN THE OCALA NATIONAL FOREST, MARION COUNTY, FLORIDA.

Guild[1]	Species	Treatment: Burn-salvage (HIBS) (n = 3)	Chop (RC) (n = 3)	Bracke (BK) (n = 3)	Mature (MF) (n = 3)
	CERAMBYCIDAE	2	—	2	2
X	*Archodontes m. melanopus* (L.)	1	—	—	1
X	*Prionus pocularis* Dalman	—	—	—	1
X	*Romulus globosus* Knull	1	—	2	—
	COCCINELLIDAE	—	3	—	—
P	*Cycloneda s. sanguinea* (L.)	—	3	—	—
	CURCULIONIDAE	7	5	2	13
H	*Agraphus bellicus* (Say)	7	3	1	12
H	*Gerstaekeria hubbardi* LeConte	—	2	—	—
H	*Hylobius pales* Boheman	—	—	—	1
H	*Sphenophorus* sp.	—	—	1	—
	ELATERIDAE	1	—	1	—
P	*Agrypnus rectangularis* (Say)	—	—	1	—
H	*Elaterid* sp.	1	—	—	—
	HISTERIDAE	1	—	—	—
P	*Histeridae*	1	—	—	—
	PHENGODIDAE	2	—	—	—
P	*Phengodes* sp.	2	—	—	—

[1]H = Herbivore; X = Xylophage; P = Predator; S = Scavenger.

TABLE 3. (CONTINUED) TOTAL NUMBER OF COLEOPTERANS CAPTURED IN EACH OF THREE TREATMENTS AND MATURE FORESTED SAND PINE SCRUB FROM OCTOBER 1991 THROUGH SEPTEMBER 1992 IN THE OCALA NATIONAL FOREST, MARION COUNTY, FLORIDA.

Guild[1]	Species	Treatment: Burn-salvage (HIBS) (n = 3)	Chop (RC) (n = 3)	Bracke (BK) (n = 3)	Mature (MF) (n = 3)
	SCARABAEIDAE	62	33	30	40
S	*Ateuchus lecontei* (Harold)	1	—	—	—
H	*Diplotaxis* sp.	12	7	5	1
S	*Peltotrupes youngi* Howden	23	2	6	28
S	*Phanaeus igneus floridanus* d'Olsoufieff	2	9	1	—
H	*Strategus antaeus* (Drury)	24	15	18	11
	SILPHIDAE	3	3	—	—
S	*Nicrophorus carolinus* (L.)	3	3	—	—
	STAPHYLINIDAE	—	1	—	1
P	Staphylinid sp. 1	—	—	—	1
P	Staphylinid sp. 2	—	1	—	—
	TENEBRIONIDAE	103	105	157	55
S	*Glyptotis cribatus* LeConte	2	—	5	3
S	*Gonwanocrypticus obsoletus* (Say)	—	1	—	—
S	*Helops* sp.	32	40	56	—
S	*Polopinus disjunctus* Kritsky	—	1	—	—

[1]H = Herbivore; X = Xylophage; P = Predator; S = Scavenger.

TABLE 3. (CONTINUED) TOTAL NUMBER OF COLEOPTERANS CAPTURED IN EACH OF THREE TREATMENTS AND MATURE FORESTED SAND PINE SCRUB FROM OCTOBER 1991 THROUGH SEPTEMBER 1992 IN THE OCALA NATIONAL FOREST, MARION COUNTY, FLORIDA.

		Treatment			
Guild[1]	Species	Burn-salvage (HIBS) (n = 3)	Chop (RC) (n = 3)	Bracke (BK) (n = 3)	Mature (MF) (n = 3)
S	*P. youngi* Kritsky	47	52	80	18
S	*Polypleurus* sp.	22	11	16	34
	TOTAL INDIVIDUALS	483	403	560	403

[1]H = Herbivore; X = Xylophage; P = Predator; S = Scavenger.

TABLE 4. MEAN (± SE) SPECIES RICHNESS, DIVERSITY, AND EVENNESS OF COLEOPTERA TRAPPED FROM OCTOBER 1991 TO SEPTEMBER 1992 IN THREE TREATMENTS AND MATURE FORESTED SAND PINE SCRUB, OCALA NATIONAL FOREST, FLORIDA.

	Richness	Diversity	Evenness
Burn-salvage (HIBS)	15.3	0.800	0.676
	(1.9)	(0.057)	(0.019)
Chop (RC)	13.7	0.783	0.691
	(1.5)	(0.061)	(0.026)
Bracke (BK)	14.7	0.763	0.655
	(0.7)	(0.018)	(0.025)
Mature (MF)	10.7	0.681	0.667
	(0.9)	(0.048)	(0.055)
P-value	0.098	0.385	0.899
F-value	3.00	1.15	0.19
df	2	2	2

between mature and successional coniferous taiga. This appears to be the case for sand pine scrub as well.

Several possibilities exist for the similarity in species composition and community structure among disturbance treatments and mature sand pine scrub. The pitfall trapping technique may have missed important species (Adis 1989), including non-terrestrial species such as many monophagous herbivores, xylophages, and mature forest-specialists. However, similar studies using pitfall traps detected differences in species composition among treatments (e.g. Lenski 1982, Baguette & Gerard 1993, Buse & Good 1993, Halme & Niemela 1993, Niemela et al. 1993). Because trap bias is consistent across treatments, comparisons using standardized trapping techniques are valid.

Differences among treatments may have been present for the first few years following disturbance but were not detected because stands were not sampled until 5-7

TABLE 5. HORN'S INDEX OF COMMUNITY SIMILARITY (R_O)[2] FOR COLEOPTERA IN THREE TREATMENTS AND MATURE FORESTED SAND PINE SCRUB, OCALA NATIONAL FOREST, FLORIDA.

	Burn-salvage (HIBS)	Chop (RC)	Bracke (BK)
Burn-salvage (HIBS)			
Chop (RC)	0.931		
Bracke (BK)	0.945	0.939	
Mature (MF)	0.913	0.821	0.874

[2]As R_o approaches one, community overlap increases.

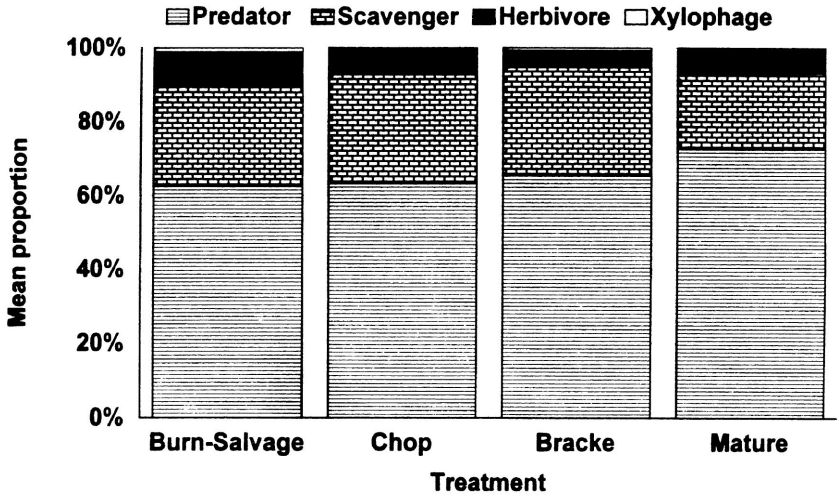

Fig. 1. Mean proportion of Coleoptera in four feeding guilds in three disturbance treatments and mature forest in sand pine scrub, Ocala National Forest, Florida.

years post-disturbance. Small stand size and time since disturbance permitted ample opportunity for recolonization of disturbance treatments by coleopterans. Nonetheless, similarity in coleopteran community composition indicates that suitable habitat exists 5-7 years post-disturbance, whether by silvicultural means or by wildfire.

Similarity of many habitat features between 5-7-year-old disturbed and mature sand pine scrub may also contribute to like coleopteran community composition among treatments. Differences in pine age, height, and density, as well as percent bare ground were the most prominent differences between MF and disturbance treatments. However, minimal differences in composition of dominant (woody) plant species exist between early and late-successional sand pine scrub or among disturbance treatments (Greenberg et al. 1995). Infertile, acid sands and low water availability render productivity and palatability of the sclerophyllous vegetation low in sand pine scrub. Although several species of herbs occur within the study area (Greenberg et al. 1995), total cover is low. These factors may partially explain the low representation of herbivorous coleopterans. Nonetheless, endemic, host-specific herbivores might be expected where plant species distribution is restricted as is sand pine or endemic herbaceous species of scrub (Deyrup 1989).

Herbaceous plant species diversity is higher in disturbed scrub than in mature forest. Among disturbance treatments, herbaceous plant community similarity, species richness, and species diversity did not differ significantly (Greenberg et al. 1995). However, disturbance treatments could differentially affect occurrences of some plant species. Trap bias against plant specialists and xylophages could lead to potential differences among treatments going undetected.

The apparent absence of forest specialists within sand pine scrub could be due to the historical prevalence of young forests due to low-frequency, high intensity wildfire (Rawlings 1933, Webber 1935, Bartram 1955, Bonan & Shugart 1989). Even in the absence of fire, sand pine stands begin to break up after about 50-70 years (Myers 1990) due to disease or structural weakness. Historically, sand pine density in the study area probably varied spatially and temporally as well; many scrubs have few to no

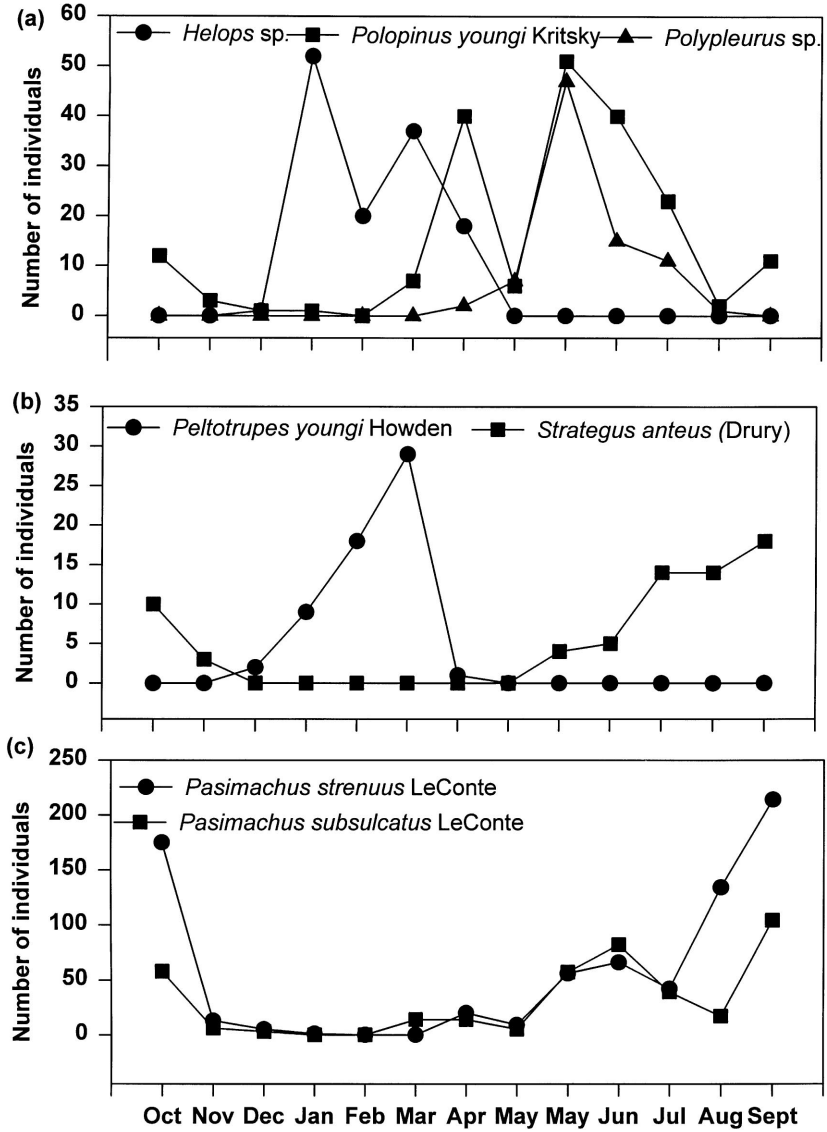

Fig. 2. Annual above-ground terrestrial activity cycles of (a) tenebrionids; (b) scarabs; and (c) carabids in the Ocala National Forest, Florida.

sand pine. Coleopterans are unlikely to have evolved into a specialized mature forest niche where habitat availability was rare or unreliable.

The predominance of carabids, tenebrionids, and staphylinids reflects both the trap bias toward ground-dwelling coleopterans and their relative abundance. The overwhelming prevalence of predaceous Coleoptera (primarily carabids) may be due

to an abundance of arthropod prey within sampled sites (C. H. G. unpublished data). Predaceous beetles may have consumed non-predaceous beetles in traps, but the additional presence of other arthropod prey reduces the likelihood that this was a significant problem.

Xylophagous species were under-represented in this study due to trap bias. Additionally, this guild may have been more abundant had HIBS not been salvage-logged. However, small-diam woody debris was available in the form of slash piles (from on-site delimbing) in HIBS and BK treatments. Woodpiles were less common in RC sites because the roller-chopping fragmented and buried woody debris (Table 2). Few snags were present in MF or in disturbance treatments.

Stand age may also account for low representation by xylophages which may have been more abundant during the first 1-2 years post-logging. The absence of pyrophyllous species in the HIBS treatment was probably due to elapsed time since fire and post-fire salvage-logging.

Monthly variation in above-ground terrestrial activity cycles suggests that trapping in all months is necessary for a complete census of coleopterans as well as for gaining an understanding of their ecology.

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TWO NEW SPECIES OF *DEUTEROSMINTHURUS* (BOURLETIELLIDAE), EPIPHYTIC COLLEMBOLA FROM THE NEOTROPICAL REGION WITH A KEY FOR THE AMERICAN SPECIES

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ABSTRACT

Two new species of *Deuterosminthurus* are described and illustrated. One is found in dry forest canopy at Chamela, Jalisco, México, and the other is associated with sugarcane at La Habana, Cuba. A key for identification of the American species is included.

Key Words: *Deuterosminthurus*, taxonomy, species key

RESUMEN

Se describen e ilustran dos nuevas especies de *Deuterosminthurus*. Una de ellas encontrada en la canopia de la selva baja caducifolia de Chamela, Jalisco, México, y la otra asociada al cultivo de la caña de azúcar en La Habana, Cuba. Se incluye una clave para la identificación de las especies del Continente Americano.

Deuterosminthurus (Börner, 1901)

Body dorsally with a depression behind middle of great abdomen. Anogenital segment strongly elongated and distinctly delimited from great abdomen. Integument finely granulated. Setae unciliated, curved and moderately long. Great abdomen with three pairs of fine and long trichobothria inserted in low papillae. Two trichobothria on each side of genital segment. Ant. IV divided into 3 - 9 secondary joints in addition to basal and apical parts. Eyes 8 + 8. Claw untoothed or armed with very small inner and lateral teeth; tunica and pseudonychia absent. Unguicular filament sharply pointed, or blunt at the tip, or knobbed. Tenent hairs clavate 3, 3, and 2. Dentes smooth, about three times the length of mucro. Mucro spoon-like with smooth dorsal edges, without mucronal seta. Secondary dimorphism in male: smaller body and head length, longer antennae and different shape of anogenital segment; clasping organ on anal segment absent. Female with subanal appendages. Type species: *Sminthurus bicinctus* Koch, 1840.

The genus has 32 named species; 8 are known from the Neotropical Region and 7 from the Nearctic Region. *Deuterosminthurus lippsoni* Snider, 1978 is recorded in both regions. It was described from Maryland and reported from Florida and California. This species was also reported from Cuba by Banasco Almentero (1987). This ge-

nus was only recently recorded from Chamela, México (Palacios-Vargas & Gómez Anaya, 1993), as described here in more detail.

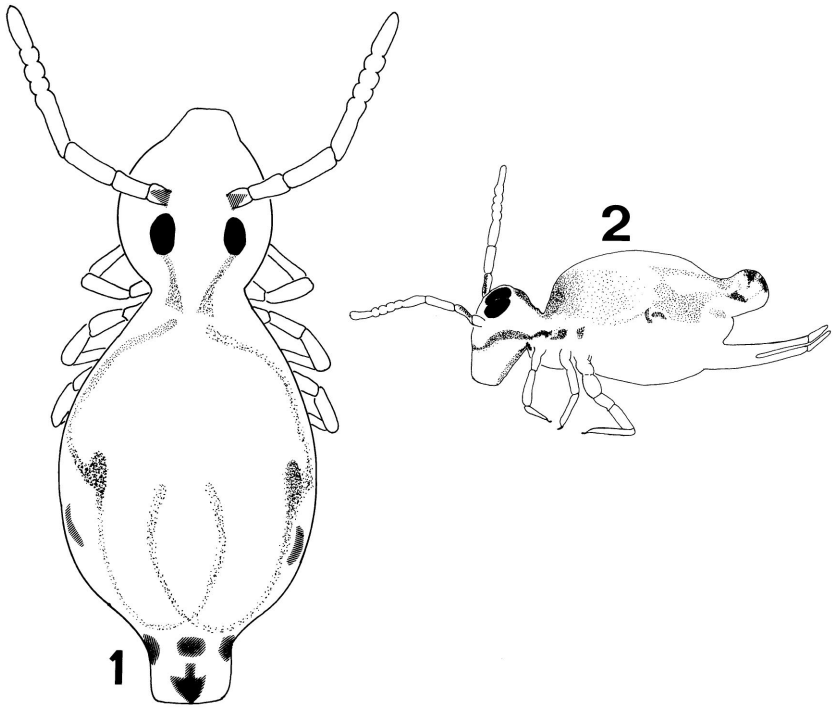
Deuterosminthurus is a cosmopolitan genus that lives in litter, epiphytic Bromeliaceae and the forest canopy. One species, *D. russatus* Maynard, 1951 is known to be part of the transitory epineuston, so it can be found in lakes and other freshwater bodies.

Deuterosminthurus delatorrei **New Species**
(Figs. 1 - 16)

DESCRIPTION

Antennae light yellow, segment I with purple pigment dorsally. Head with two bands of pigmentation (Figs. 1 and 2), one posterior to eyes and one laterally; genae with purple pigment extending to occiput. Body yellow with purple bands and patches forming a "W". Abd. VI with two small dark purple patches. Legs and furcula without pigmentation.

Eyes 8 + 8; ocellus D half the diam of H (Fig. 3). Antennal segments female (*n*=9) ratio 1:1.5:2:1:4.9 (Fig. 4); male (*n*=2) 1:2:2.5:5.7. Ant. IV subannulated into four intermediates. Apical bulb simple; one sensorial small organ and one dorso-external microsensillum (Fig. 5). Ratio head-antenna: female, 1:1.3; male, 1:1.4. Ant. III with



Figs. 1 and 2. *Deuterosminthurus delatorrei* sp. nov. 1. Dorsal distribution of pigments. 2. Habitus lateral view.

subapical sensory rods lying in shallow depressions; an accessory sensory rod slightly oblique and posterior to sensory rods; setae normal (Fig. 6). Thoracic segmentation not distinct. Metatrochanters without oval organs. Metafemora with two posterior setulae. Tibiotarsi of the pro- and mesolegs with three well-developed, appressed, clavate tenent hairs; meta-tibiotarsi with two tenent hairs (Figs. 7 - 11). Pretarsus with anterior setulae. Unguis lanceolate with inner tooth short and a small lateral tooth. Unguiculus with a strong bristle, tapering to a strong knob (Figs. 9, 11). Ratio unguiculus: unguis = 1:0.9. Sacs of ventral tube tuberculate. Rami of tenaculum tridentate (Fig. 12); anterior corpus with three apical setulae. Manubrium with 12 dorsal setae. Dens with 5 vental setae, 6 lateral and 19 others, one of them in dorsal position similar to a bothriotricum (Fig. 13). Mucro with rachis fused to lateral lamellae in a spoon shape, rachis forming a clear tip distally. Ratio mucro: dens = 1:2.7. Anal papillae with normal curving setae; female subanal appendage setiform (37 µm), tip may be acuminate or with 2 - 4 teeth (Fig. 14). Setae of head and body short and curving. Male with eugenital setae longer than circumgenital setae (Fig. 15). Maximum size of female (*n*=9) 0.7 mm and male (*n*=2) 0.47 mm. Head chaetotaxy as in Fig. 16. Male similar to female except for the lack of subanal appendages and smaller size.

TYPE LOCALITY

Cuba: La Habana: Boyeros. Experimental sugarcane field.

TYPE MATERIAL

Holotype ♀ on slide, two ♂♂ paratypes and eight ♀♀ paratypes on slides. Two paratypes will be kept at the Facultad de Biología, Universidad de La Habana, the holotype and other paratypes at Facultad de Ciencias, UNAM. Type material data: sugar cane field, 80m altitude, yellow traps, 28-IV-1992, 4-VI-1992, V. González, M. Díaz and D. Prieto colls.

VARIATION

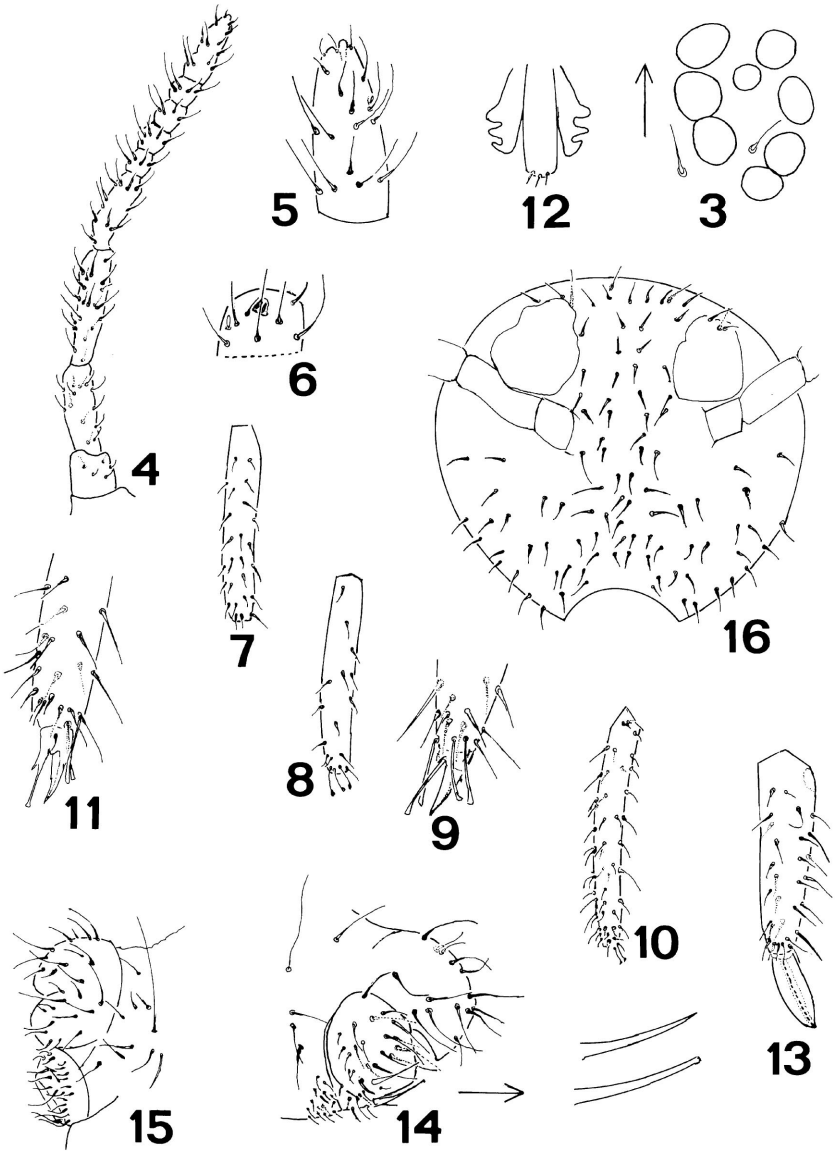
The number of teeth on subanal appendage (*n*=11) varied as follows (frequency in parentheses): one (1), two (5), three (3), and four (2). In the same specimen the number of teeth may vary from side to side. One bifid seta was observed on a trochanter. Another specimen had one extra ventral dental seta.

ETYMOLOGY

This species is named after Dr. Salvador de La Torre, Cuban entomologist.

DISCUSSION

The new species is very similar to *D. lippsoni* Snider, 1978, which shares the presence of a heavily knobbed unguiculus. Other species with a knobbed unguiculus are *D. xeromorphus* Snider, 1978 and *D. wexfordensis* (Snider, 1969). *D. lippsoni* differs in having five subsegments of antennal IV (after the original description) while in *D. delatorrei* it has four. The only reduced ocellus in the new species is "D". *D. lippsoni* has an oval organ in the metatrochanter, which is lacking in *D. delatorrei*. *D. lippsoni* has



Figs. 3 - 16. *Deuterosminthurus delatorrei* sp. nov. 3. Right eyepatch. 4. Antennal segments I to IV. 5. Ant. IV distal portion. 6. Ant. III distal portion. 7. Tibiotarsus II external view. 8. Tibiotarsus II internal view. 9. Foot complex of leg II. 10. Tibiotarsus III. 11. Foot complex of leg III. 12. Tenaculum. 13. Dens and mucro. 14. Female, anal and genital region, with subanal appendages enlarged, lateral view. 15. Male, anal and genital region. 16. Head chaetotaxy.

more pigmentation in a very different pattern. Subanal appendages in *D. delatorrei* are thicker and apically toothed.

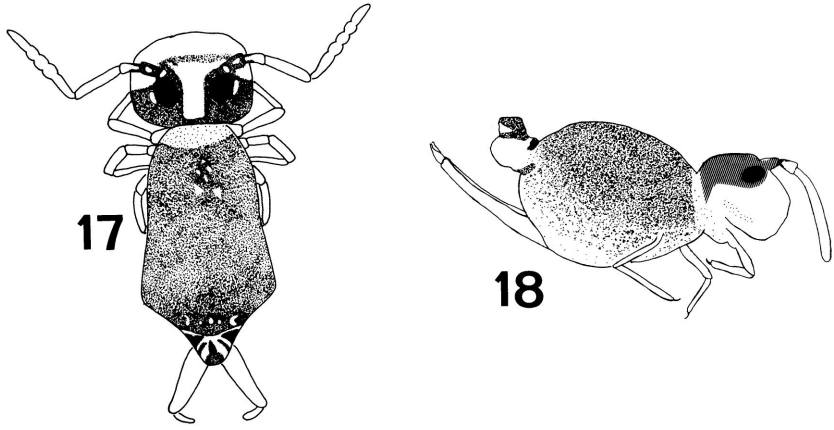
This species occurs exclusively in epiphytic environments. It was never found in soil and litter samples taken monthly during a two-year study of sugarcane. This species was first caught when yellow traps were used.

Deuterosminthurus maassius **New Species**
(Figs. 17 - 29)

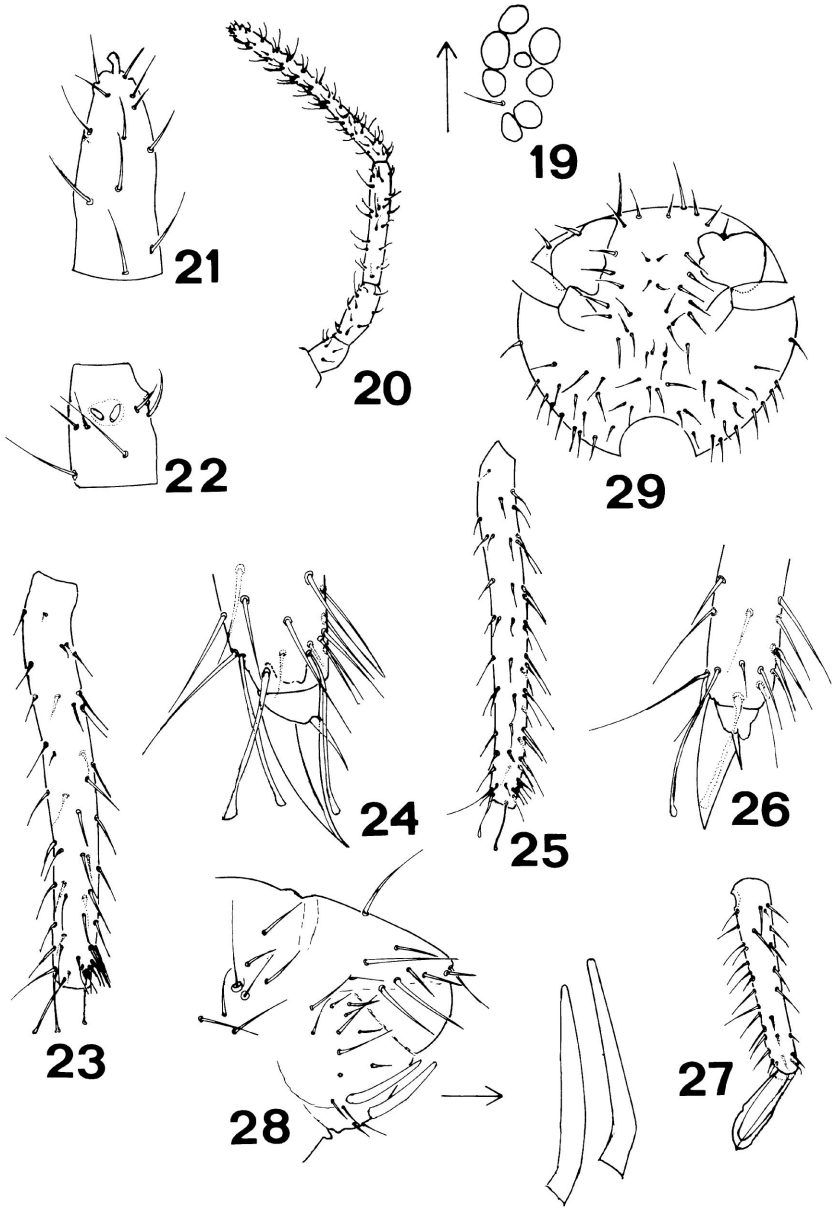
DESCRIPTION

Antennae light yellow, segment I with dark purple pigment. Head with ocular patches and posterior region with pigmentation (Figs. 17, 18). Great abdomen very dark, except for a clear area in the middle thoracic region. Anal papilla with only dorsal pigmentation dark purple; ventrally body is light, lacking of dark purple. Legs and furcula lack pigmentation.

Eyes 8 + 8; ocellus D half the diam of H; F and G a little smaller than H (Fig. 19). Antennal segments female, (*n*=8) ratio of 1:1.7:3:1:5.4 (Fig. 20). Ant. IV slightly subannulated into three intermediates. Apical bulb simple; one small sensorial organ and one microsensilla dorso-external (Fig. 21). Ratio head: antenna as 1: 1.6. Ant. III with subapical sensory rods lying in shallow depressions; accessory sensory rod slightly oblique and posterior to sensory rods; with ventral microsensillae (Fig. 22). Some setae on Ant. I and II are long and thick. Thoracic segmentation not distinct. Metatrochanters with oval organs. Metafemora without posterior setulae. Tibiotarsi of the pro- and mesolegs with 3 strong, appressed, clavate tenent hairs; meta-tibiotarsi with 2 tenent hairs (Figs. 23, 25). Pretarsus with an anterior setula. Unguis lanceolate with a small apical inner tooth and small lateral teeth. Unguiculus lanceolate ending in an acuminate bristle (Figs. 24, 26). Ratio unguiculus: unguis = 1:2.3. Sacs of ventral tube tuberculate. Rami of tenaculum tridentate; corpus with 3 apical setulae. Manubrium with 12 dorsal setae. Dens with 3 ventral setae, 6 lateral and 13 others, one of them in dorsal position similar to a bothriotricum (Fig. 27). Mucro with rachis fused to lat-



Figs. 17 and 18. *Deuterosminthurus maassius* sp. nov. 17. Dorsal distribution of pigment. 18. Habitus lateral view.



Figs. 19 - 29. *Deuterosminthurus maassius* sp. nov. 19. Right eyepatch. 20. Antennal segments I to IV. 21. Ant. IV distal portion. 22. Ant. III distal portion. 23. Tibiotarsus II. 24. Foot complex of leg II. 25. Tibiotarsus III. 26. Foot complex of leg III. 27. Dens and mucro. 28. Female, anal and genital region, with subanal appendages enlarged, lateral view. 29. Head chaetotaxy.

eral lamellae in a spoon shape, edges somewhat undulating, rachis forming a thin tip at the end of the mucro. Anal papillae with normal curving setae; female subanal appendage large and very thick, apically rounded (67 µm long, 7.8 µm wide) (Fig. 28). Setae of head and body long and thick, some of them appear as spines (Fig. 29). Two pairs of anal setae are very thick. Maximum size of female (*n*=8): 1.22 mm. The male is unknown.

TYPE LOCALITY

Mexico: Jalisco: Chamela, Estación de Biología, Instituto de Biología, Universidad Nacional Autónoma de México.

TYPE MATERIAL

Holotype ♀ on slide, eight ♀♀ paratypes on slides. Two paratypes will be kept at the Institute of Biology, UNAM and two at Chamela Field Station, the remainder at Facultad de Ciencias, UNAM. Data of type material: tropical dry forest,40 m altitude, fogging, 18-VIII-1992, A. Pescador, A. Rodríguez Palafox and J. A. Gómez Anaya colls.

VARIATION

One specimen had four intermediate subdivisions on Ant. IV.

ETYMOLOGY

This species is dedicated to Dr. Manuel Maass, Centro de Ecología, UNAM for his contributions to tropical dry forest ecology.

DISCUSSION

Deuterosminthrus maassius sp. nov. is similar to *D. tristani* Denis, 1933 from Costa Rica. The new species differs in having no pigmentation on the antennae and a different color pattern. Head chaetotaxy is similar, with four small setae on the frons; however, *D. maassius* lacks tubercles with setae. The subanal appendage of female *D. tristani* is bifid, whereas that of *D. maassius* is thick and apically rounded. Compared to the drawings of Denis (1933), there seems to be a different ventral dental chaetotaxy. Mucronal edges of the new species are somewhat undulate, while in *D. tristani* species they are smooth. Both species share the presence of large stout setae, spine-like on the head and body; most of the species in the genus have small to moderate and thin setae.

We collected soil and litter samples (*n*=960) during a two-year study at Chamela and never found *D. maassius* sp. nov. However, several specimens were caught with Malaise traps, and also with the aid of a fogger and contact insecticide. Therefore this species is apparently also associated with epiphytes. In September 1992, we fogged the canopy in an area covering 100 m^2. Among other arthropods we collected more than 1,013,000 specimens of Collembola. We identified 16 species of Collembola, including *D. maassius* sp. nov. However the great majority (98%) belonged to *Salina banksi* MacGillivray, 1894.

KEY TO SPECIES OF THE GENUS *DEUTEROSMINTHURUS* (BÖRNER, 1901) FROM THE AMERICAN CONTINENTS *

1. Needle of unguiculus not apically knobbed 2
   Needle of unguiculus knobbed 3

2. Dens with one subapical ventral seta; setae on head and body thick, spine-like (Costa Rica)..... *D. tristani* Denis
   Dens with several subapical ventral setae (3-6); setae on head and body thin and short 4
3. Ant. IV with 4-5 intermediate subsegments 5
   Ant. IV with 7 - 15 intermediate subsegments 10
4. Unguiculus as long as ventral edge of unguis 12
   Unguiculus short, 1/3 - 1/2 as long as ventral edge of unguis 6
5. Dens with 5 ventral setae; female appendage with 2 (1-4) teeth (Cuba)..... *D. delatorrei* sp. nov.
   Dens with 6 ventral setae; female appendage without any teeth (USA and Cuba)..... *D. lippsoni* Snider
6. Dens with 3 ventral setae 7
   Dens with 5 - 6 ventral setae 8
7. Dens with 3 very short ventral setae; Ant. IV with 5-7 intermediate subsegments (USA) ..... *D. russatus* Maynard
   Dens with 3 long ventral setae; Ant. IV with 3 intermediate, poorly defined subsegments; head and body with thick spine- like setae (México)..... *D. maassius* sp. nov.
8. Dens with 5 ventral setae and 6 intermediate subsegments (Brazil) ..... *D. salinensis* Arlé
   Dens with 6 ventral setae 9
9. Ant. IV with 6 subsegments; tip of female appendage without any teeth; tibiotarsus with spines (Brazil)..... *D. richardsi* Arlé
   Ant. IV with 6-9 subsegments; female appendage spatulate with 7 teeth; without spines on tibiotarsus III (USA) ..... *D.lurida* Snider
10. With ciliated setae on body; very small species (0.75 mm) (USA)..... *D. xeromorphus* Snider, 1978
    With smooth setae on head and body; larger species (1.0-1.3 mm) 11
11. Dens with 6 ventral setae; unguiculus with a very long tooth; female appendage spatulate with 5 teeth (USA) ..... *D. validentatus* Snider
    Dens with 5 ventral setae; unguis with small tooth; tip of female appendage without teeth ..... (USA) *D. wexfordensis* (Snider)
12. Ant. IV with 6 intermediate subsegments (Brazil) ..... *D. aueti* Arlé
    Ant. IV with 14 - 15 intermediate subsegments (USA)..... *D. nonfasciatus* Snider

*Dubious records and inaccurately described species are not included in this key.

Endnote

Specimens of *Deuterosminthurus* from Cuba were collected by Lic. Magaly Díaz and Dr. Dania Prieto; those from Chamela were collected by Dr. Alfonso Pescador, M. Sc. Alicia Rodríguez and José A. Gómez Anaya. We are gratefull to all of these collaborators. Proyecto DGAPA IN2078/91 y Convenio de Intercambio Académico México-Universidad de La Habana.

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GENETIC FINGERPRINTING OF THE SCREWWORM (DIPTERA:CALLIPHORIDAE) INFESTATION IN NORTH AFRICA BY MITOCHONDRIAL DNA MARKERS

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ABSTRACT

A screwworm sample, *Cochliomyia hominivorax* (Coquerel), collected from Libya in 1990, during the final phase of the eradication program, was analyzed for mitochondrial DNA (mtDNA) RFLP patterns. The goal was to determine whether these fertile flies represented reintroduction from either a new source or contaminants in the shipments of sterile flies from the Mexican screwworm factory, (used for eradication of flies in Libya) or progeny of the original introduction in 1988. Samples of laboratory-strains originating from South America, Mexican factory, Costa Rica, and a 1989 collection from Libya were also analyzed. These patterns were compared with the previously published patterns of 30 laboratory strains originating from Mexico, Central America and Jamaica and earlier sample of flies from Libya. The restriction patterns (with *Eco*RV, *Fnu*4HI, *Hind*III, *Hpa*I, *Msp*I, *Scrf*I and *Sst*I) of 1990-Libyan screwworm were similar (mitochondrial similarity, F=0.97) to those of South American flies but different from those of Mexico (F=0.53), Jamaica and Central America (F=0.71). The *Hpa*I and *Ssp*I digests of DNA amplified by primer flanking mitochondrial CO-I to CO-II region produced patterns which were similar in Libyan and South American flies but different from those of Mexican and Costa Rican samples. These data confirmed that the flies collected in Libya in 1990 did not originate from Mexico, Central America or Jamaica. Our data suggest some region(s) of South America could be the likely source of screwworm infestation in North Africa. However, it would be necessary to analyze several geographical samples from South America to confirm this tentative conclusion.

Key Words: Screwworm, *Cochliomyia hominivorax*, PCR, mitochondrial DNA RFLP, geographical variation

RESUMEN

Fueron analizados los patrones RFLP de DNA mitocondrial (mtDNA) de una muestra de gusanos barrenadores del ganado, *Cochliomyia hominivorax* (Coquerel), colectada en Libia en 1990 durante la fase final del programa de erradicación. El objetivo fue determinar si las moscas habían sido reintroducidas de una nueva fuente o

si eran contaminantes en los envíos de moscas estériles de la fábrica de gusanos barrenadores del ganado de México (usados para la erradicación de las moscas en Libia) o si eran parte de la progenie de la introducción original de 1988. También fueron analizadas muestras de cepas de laboratorio originales de América del Sur, de la fábrica de México, de Costa Rica, y de una colección de Libia. Estos patrones fueron comparados con los patrones previamente publicados de 30 cepas de laboratorio de México, America Central y Jamaica y con muestrtas anteriores de moscas de Libia. Los patrones de restricción (con *Eco*RV, *Fnu*4HI, *Hind*III, *Hpa*I, *Msp*I, *Scrf*I y *Sst*I) de gusanos de tornillo de Libia de 1990 fueron similares (similitud mitocondrial, F=0.97) a aquellos de las moscas de América del Sur, pero diferentes de los de México (F=0.53), Jamaica y América Central (F=0.71). Los análisis de restricción de DNA con *Hpa*I y *Ssp*I amplificados por "primer flanking" a la región mitocondrial CO-I a CO-II originaron patrones similares a los de las moscas de Libia y América del Sur y diferentes de los de las muestras de México y Costa Rica. Estos datos confirman que las moscas colectadas en Libia en 1990 no se originaron en México, América Central o Jamaica. Nuestros datos sugieren que alguna región o regiones de América del Sur podrían ser la fuente de la infestación de Africa del Norte. Sin embargo, sería necesario analizar varaias muestras geográficas de América del Sur para confirmar esta conclusión tentativa.

The New World screwworm (NWS), *Cochliomyia hominivorax* (Coquerel) (Diptera:Calliphoridae), is a major agent of myiasis of livestock and wildlife in the New World (Knipling & Rainwater 1937). Originally, it was widely distributed from the southern U.S. to northern Chile. However, it was eradicated in the United States by August, 1982, and in Mexico in February, 1991, (FAO 1992) by the sterile insect technique (SIT), as described by Knipling (1955). In March, 1988, NWS were detected in North Africa (Libya) causing wound infestations in livestock and humans. This caused great concern that the screwworm might spread to Europe, throughout Tunisia and Egypt, and up the Nile into Sub-Saharan Africa. In 1991, the United Nations Food and Agriculture Organization (FAO) declared eradication of screwworm from Libya by the sterile insect technique.

The source of the 1988 screwworm infestation in Libya is not known. Taylor et al. (1991) compared the mitochondrial DNA (mtDNA) restriction pattern of the Libyan flies with the previously published patterns (Roehrdanz, 1989) of 30 strains originating from Mexico, Central America and Jamaica. They concluded that the 1989 collection of Libyan flies had a unique mitochondrial genotype and that the 1988 infestation in Libya did not originate from Jamaica, Mexico or Central America.

During May, 1991, we received a sample of flies from Libya (collected in November, 1990, near the end of the eradication program). We also received flies from South America (Brazil) and Costa Rica. We analyzed mitochondrial DNA of these flies to determine whether or not: 1) the latest sample from Libya represented a new introduction, 2) PCR-based DNA markers could be used to distinguish Libyan flies from South American, Central American, Mexican and Jamaican flies, and 3) there were any genetic differences between Costa Rican samples collected in 1986 and 1991.

Materials and Methods

Collection of Samples

The 1990 Libyan sample was from a strain which originated from three egg masses collected from wounds on sheep near Tripoli, Libya, in November of 1990, and

that were maintained in Libya for five generations. A sample of pupae was transported from Libya to Fargo and the adults were used for mtDNA and PCR studies. Frozen samples of the 1989 Libyan flies (LIB-89) were also analyzed. These flies originated from a laboratory strain established from two eggs masses collected near Tripoli in October, 1989. Flies were frozen for mtDNA analysis each generation up to 15 generations. The South American sample (BRA-90) was collected in August, 1990, by exposing a wounded cow to feral flies in Rio de Janeiro, Brazil. About 500 larvae were collected from the wound, reared to pupae and transported to Fargo. The adults were combined and frozen samples from the ensuing F_5 and F_{10} generations were used for DNA analysis. The Costa Rican sample of 1991 (COR-91) was from a composite laboratory strain. It originated from eggs laid by twenty-two individual flies collected on liver. Of these isofemale lines, only sixteen were established successfully in the laboratory. These were later combined to establish a composite strain. After 2-3 generations of culture in the laboratory, adults were frozen for mtDNA analysis. Flies originating from the 1986 collection from Costa Rica (COR-86) were analyzed earlier by Roehrdanz (1986). We compared mtDNA data of COR-91 with that of COR-86. The OW-87 strain was used as a reference standard for comparison of our results with those published by Roehrdanz (1989). This strain is currently being mass-produced in Mexico for use in the sterile insect release program in Central America. The OW-87 was also released in Libya. This strain originated from egg masses collected in the vicinity of Orange Walk, Belize, in October, 1986 (refer to Taylor et al. 1991).

Restriction Analysis of mtDNA.

Total cellular DNA from individual flies was isolated by the method of Boyce et al. (1989) with minor modifications. Individual flies were homogenized in 700 µl of CTAB buffer (0.1 M tris HCl, pH 8.0, 1.4 M NaCl, 0.02 M EDTA, 2% CTAB - hexadecyl trimethylammonium bromide, 0.2% 2-mercaptoethanol), incubated at 65°C for 45 minutes and centrifuged. The supernatant was extracted with an equal volume of chloroform:isoamyl alcohol and DNA was precipitated by adding 2/3 vol of isopropanol. The DNA pellet was washed with cold 70% ethanol, vacuum dried and dissolved in 50 µl 1X TE (10 mM tris, 1 mM EDTA, pH 8.0) containing 10 µg/ml DNAse free RNAse A.

DNA samples were digested separately with 15 restriction endonucleases and electrophoresed in 1% agarose gels in TBE buffer. Two molecular size markers, 1 Kb ladder and lambda *Hind*III (purchased from BRL) were used in each gel for estimation of size of restriction fragments. The DNA gels were blotted on to nylon membranes according to Reed & Mann (1985). Purified mtDNA from laboratory reared screwworm pupae of OW-87 (courtesy of Dr. Roehrdanz) was used to probe Southern blots. The hybridization mixture contained ^{32}P labeled probe and molecular size markers in 0.5 M phosphate buffer (pH 7.0), 1 mM EDTA, 1% BSA and 7% SDS. After overnight hybridization at 65°C, membranes were washed and exposed to X-ray film. The mitochondrial similarities (*F*) among geographical samples, based on the proportion of identical fragments, were calculated by the method of Nei & Li (1979).

PCR Amplification.

Total DNA prepared from individual adults served as a source of mtDNA template for the PCR. We used sequences from conserved regions spanning 12SrRNA, 16SrRNA, isoleucine t-RNA, CO-I and CO-II subunit genes of *Drosophila* (Simon et al. 1991) and the honeybee (Hall & Smith 1991) as primers (Table 1). These oligonu-

TABLE 1. LIST OF PRIMERS USED TO AMPLIFY CERTAIN REGIONS OF MITOCHONDRIAL GENOME OF SCREWWORM, *COCHLIOMYIA HOMINIVORAX*, BY POLYMERASE CHAIN REACTION.

Primer Set No.		Sequence	Ref. Fly Sequence
1	►	5' AAA CTA GGA TTA GAT ACC CTA TTA T 3'	12sai (Simon et al. 1991)
	◄	5' ATG TTT TTG ATA AAC AGG CG 3'	16sa (Simon et al. 1991)
2	►	5' CGC CTG TTT ATC AAA AAC AT 3'	16sar (Simon et al. 1991)
	◄	5' CTC CGG TTT GAA CTC AGA TC 3'	16sbr (Simon et al. 1991)[1]
3	►	5' ATT TAC CCT ATC AAG GTA A 3'	t-Iso (Simon et al. 1991)
	◄	5'CGG GCG ATG TGT ACA TAA TT 3'	12sfi (Simon et al. 1991)
4	►	5' ATT TAC CCT ATC AAG GTA A 3'	t-Iso (Simon et al. 1991)
	◄	5' AGG GTA TCT AAT CCT AGT TT 3'	12sair (Simon et al. 1991)
5	►	5' TTG ATT TTT TGG TCA TCC AGA AGT 3'	CO-I (Hall & Smith, 1991)
	◄	5'GAT CAA TAT CAT TGA CC 3'	CO-II (Hall & Smith, 1991)

[1]Modified 16sbr

cleotide primers were synthesized (National Biosciences) and used for amplifications of DNA segments. The PCR was performed as described by Simon et al. (1991). Each reaction volume was 100 µl and contained about 0.1 µg of total cellular DNA, 10 µl PCR buffer (10X), 150 nmole $MgCl_2$, 20 pmole of each primer, 20 nmole each of dATP, dCTP, dGTP and dTTP, and 2.5 units Taq polymerase. The reaction profile consisted of 2 cycles of 95°C for 2 min, 50°C for 2 min, 72°C for 4 min; 32 cycles of 93°C for 1 min, 50°C for 1 min, 72°C for 4 min, and a final cycle of 93°C for 1 min, 50°C for 1 min and 72°C for 10 min.

Aliquots (10 µl) of PCR product were digested with eight enzymes (*Eco*RV, *Fnu*4HI, *Hind*III, *Hpa*I, *Msp*I, *Scrf*I, *Sst*I, *Ssp*I). The digests along with a molecular size marker (∅X174 *Hae*III digest) were electrophoresed in 3.0% agarose and stained with ethidium bromide. The sizes of fragments were estimated against the molecular size marker.

RESULTS

Mitochondrial DNA Restriction Patterns

Data in Table 2 show the mitochondrial DNA restriction fragments observed with 15 enzymes. Table 3 and Figs. 1 to 5 summarize the restriction fragment patterns in LIB-89, LIB-90, BRA-90 and COR-91 strains. The designations for restriction patterns correspond to those of Roehrdanz (1989). When our estimates of the sizes of one or more fragments in a specific pattern differed slightly (500 bp or less) from those reported by Roehrdanz (1989), we maintained the same letter designation but indicated it by parenthesis. Three new patterns, not reported in earlier studies (Roehrdanz 1989, Taylor et al. 1991) are indicated with asterisks (Tables 2 and 3).

The three new patterns included: pattern F for *Msp*I in LIB-89, LIB-90 and BRA-90 (Figs. 1 & 2); G for *Hind*III in COR-91 (Fig. 3); and C for *Pvu*II in BRA-90 (not shown). These three patterns were not reported before in LIB-89 (Taylor et al. 1991)

TABLE 2. ESTIMATES OF FRAGMENT SIZES OF MITOCHONDRIAL DNA RESTRICTION PATTERN OBSERVED FOR EACH ENZYME IN GEOGRAPHICAL SAMPLES OF SCREWWORM. FOR OTHER PATTERNS NOT SHOWN HERE, REFER TO ROEHRDANZ (1989).

Enzyme	Haplotype[1]	Restriction Fragment Sizes (Kb)
*Eco*RI	A	9.0, 3.5, 1.5, 1.2, 1.05
*Hind*III	A	6.8, 4.2, 3.3, 0.52, 0.5, 0.45
	D	6.8, 4.0, 3.8, 0.52, 0.5, 0.45
	G[1]	5.5, 3.2, 2.8, 1.3, 0.52, 0.5, 0.45
*Hae*III	A	7.5, 4.8, 2.0, 1.1, 0.8
	B	7.5, 7.0, 1.1, 0.8
*Msp*I	A	5.0, 4.9, 4.2, 1.4
	B	6.1, 5.0, 4.2
	F[1]	4.9, 4.8, 4.2, 1.4, 0.3, 0.2
*Eco*RV	A	6.6, 5.0, 2.9, 1.5
	B	6.6, 5.0, 4.5
*Pvu*II	A	7.8, 3.0, 2.9, 0.87, 0.57
	C[1]	8.8, 3.0, 2.9, 0.87, 0.57
*Hpa*I	A	9.5, 6.5
	B	16
*Sst*I	A[2]	9.0, 6.0, 0.7
	B[2]	6.4, 5.2, 3.8, 0.7
*Xho*I	A	16
*Xba*I	A	16
*Pst*I	A	16
*Fnu*dII(BstuI)	A	13, 2.7
*Fnu*4HI	A	2.7, 2.6, 2.1, 1.9, 1.8, 1.4, 1.3, 0.8, 0.73, 0.4
	C	2.7, 2.6, 2.1, 1.9, 1.4, 1.3, 1.2, 0.8, 0.73, 0.6, 0.4
*Sau*96I	A	5.8, 4.7, 2.0, 1.6, 1.1, 0.4, 0.3
*Scr*FI	A	5.8, 4.8, 2.6, 1.4, 0.85, 0.75
	C	8.2, 4.8, 1.4, 0.85, 0.75

[1]Indicates that these haplotypes have not been reported in previous publications (Roehrdanz, 1989, Taylor et al., 1991).
[2]The haplotypes underlined indicate that fragment sizes are different from those reported in the previous publications.

or in thirty screwworm lines (Roehrdanz 1989). Patterns A and F of *Msp*I were not distinguishable (Fig. 1) until the gels were run for a relatively longer period of time (Fig. 2). In gels run for normal time (when the 0.6 Kb marker band of the molecular size marker, lambda *Hind*III, approaches close to the anodic end of the gel), the two *Msp*I fragments, 4.9 and 4.8 Kb of the pattern A appeared as a single band as did the 5.0 and 4.9 Kb fragments of pattern F (Fig. 1). These bands are better resolved in long-run gels as shown in Fig. 2. The other patterns shown in Figs. 4 and 5 include patterns A and B of *Sst*I, A of *Sau*96I, *Pvu*II and *Eco*RI, A and B of *Hpa*I, and A and B of *Eco*RV.

TABLE 3. COMPARISON OF MTDNA RESTRICTION GENOTYPES IN GEOGRAPHICAL SAMPLES OF SCREWWORM.

	Haplotypes in Screwworm Populations[1,2]					
Enzyme	LIB-91	LIB-89	BRA-91	OW-87	COR-91	COR-86
*Eco*RI	A	A	A	A	NT	A
*Hind*III	D	D	D	A	D,G*	A
*Hae*III	B	B	B	B	A	A
*Msp*I	F*	F*	F*	A	A	B
*Eco*RV	A	A	A	B	A	A
*Pvu*II	A	A	A,C*	A	A	A
*Hpa*I	B	B	B	A	NT	A
*Sst*I	(B)	(B)	(B)	(A)	(A),(B)	A
*Xho*I	A	A	A	A	A	A
*Xba*I	A	A	A	A	A	A
*Pst*I	A	A	A	A	A	A
*Fnu*dII(BstuI)	A	A	A	A	A	A
*Fnu*4HI	C	C	C	A	A	A
*Sau*96I	A	A	A	A	A	A
*Scrf*I	C	C	C	A	C	C

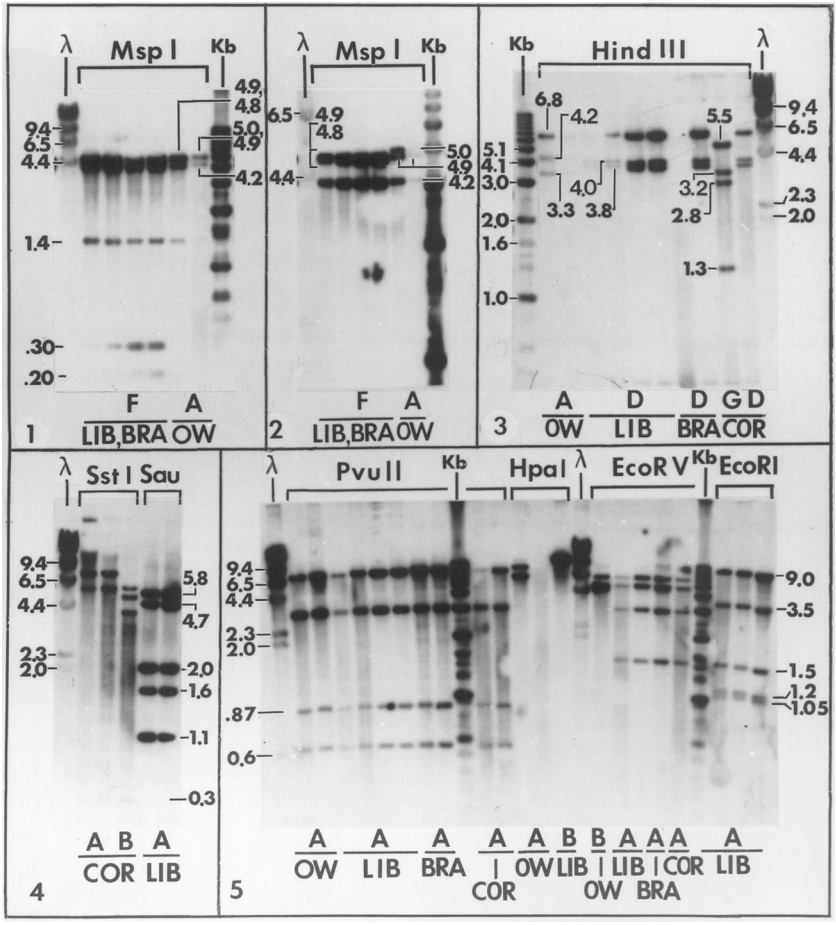
[1]The asterik (*) indicates that these haplotypes have not been reported in previous publications (Roehrdanz, 1989; Taylor et al., 1991).

[2]The haplotypes in parenthesis indicate that fragment sizes are different (refer to Table 2) from those reported in the previous publications. The data on CR-86 is from Roehrdanz (1989).

The mtDNA restriction patterns in Libyan flies for 7 (47%) enzymes (*Eco*RV, *Fnu*4HI, *Hind*III, *Hpa*I, *Msp*I, *Scrf*I and *Sst*I) were similar to those of South American (BRA-90) flies (mitochondrial similarity, F=0.97). These patterns were different from those of flies from Mexico, Jamaica and Central America. The South American sample was polymorphic for *Pvu*II sites (patterns A and C), whereas, the Libyan samples (LIB-89 and LIB-90) were fixed for pattern A. Similarly, COR-91 was polymorphic for *Hind*III (pattern D and G) and *Sst*I (patterns A and B). The COR-91 flies differed from Libyan (F=0.71) and South American flies (F=0.69) with *Fnu*4HI, *Hae*III and *Msp*I. There were more differences in restriction endonuclease recognition sites between the laboratory-reared Mexican strain and the Libyan (F=0.53) and South American (F=0.52) flies than between the latter two and flies from Costa Rica (COR-91). The patterns obtained for *Bstu*I, *Eco*RI, *Pst*I, *Pvu*II, *Sau*96I, *Xba*I and *Xho*I were not diagnostic for any population.

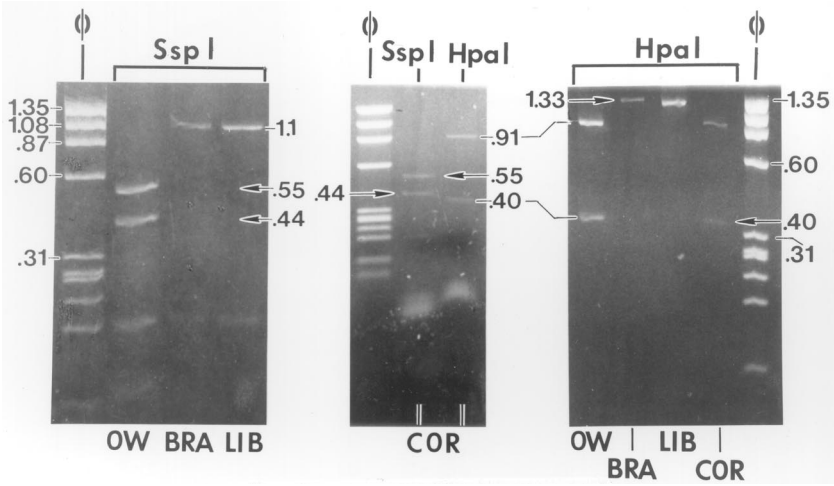
PCR Amplified DNA Restriction Patterns

The results in Table 4 show the fragments obtained by digestion of amplified DNA samples. Of the 8 restriction enzymes tested on the DNA samples amplified by primer #5 (Table 1), flanking region between CO-1 and CO-II, only two (*Hpa*I and *Ssp*I produced diagnostic patterns (Figures 6 to 8). The patterns obtained with *Hpa*I and *Ssp*I were similar in Libyan and South American flies (Fig. 6). These patterns were differ-



Figures 1-5: **1 & 2**) The mtDNA restriction fragment size differences between the *Msp*I A pattern of Mexican (OW) and the pattern F of Libyan (LIB) and South American (BRA) flies. Fragments, 5.0 and 4.9 bands of the pattern A and 4.9 and 4.8 of the pattern F can only be distinguished in Fig. 2 (from long-run gel) but not in Fig. 1 (gel run for normal period of time). Kb ladder (KB) and lambda *Hind*III digest were used as molecular size markers; **3**) *Hind*III restriction patterns showing the type A in Mexican (OW) the type D in Libyan (LIB) and South American (BRA) and types G and D in Costa Rica (COR); **4**) The patterns A and B of *Sst*I in Costa Rica (COR) and the pattern A of *Sau*96I in Libyan (LIB) flies; **5**) Restriction patterns for *Pvu*II, *Hpa*I, *Eco*RI and *Eco*RV.

ent from those obtained in Costa Rican and Mexican samples. *Hpa*I produced 2 fragments (0.91 and 0.38 Kb) in Costa Rican and Mexican strains but one uncut fragment (1.37 Kb) each in Libyan and South American strains. *Ssp*I produced 4 fragments (0.55, 0.44, 0.15, 0.08 Kb) each in Costa Rican and Mexican strains but 3 fragments (1.08, 0.15, 0.08 Kb) each in Libyan and South American strains. The patterns obtained with six other enzymes were similar in all the 4 samples tested. The digestion



Figures 6-8. Restriction patterns obtained by digestion of DNA samples amplified by PCR primer pair 5 (see Table 1). *Hpa*I and *Ssp*I patterns of LIB and BRA are different from those of OW and COR.

of DNA amplified by primer #1 flanking region between 12SrRNA and 16SrRNA region and primer #2 flanking internal sequences within 16SrRNA gene (Table 1) produced similar patterns for 8 restriction enzymes in flies from the four populations. The bands of DNA amplified by primers #3 and #4 were either weak or not reproducible.

Discussion

Our first goal was to determine whether the LIB-90 sample (collected toward the end of the screwworm eradication program in Libya) represented a new infestation. Our data on mtDNA RFLP and the PCR-amplification patterns showed no evidence for new infestation(s). The mitochondrial genome of LIB-90 was almost identical (F=0.97) to that of LIB-89. However, occasional reinfestation from the original geographical source cannot be ruled out.

Roehrdanz (1989) identified 16 mitochondrial genotypes in 30 laboratory lines originating from Mexico, Jamaica and Central America. Although restriction studies on LIB-89 by Taylor et al. (1991), did not reveal any new patterns, the composite haplotype of LIB-89 differed from those 16 reported in Jamaican, Mexican and Central American flies by Roehrdanz (1989). They designated this new haplotype as 17 and determined the cladistic relationships by the unrooted dendrogram. Although our results on restriction patterns (of LIB-89 and LIB-90 strains) for *Msp*I and *Sst*I differed (due to the improvement in resolution of fragments by our electrophoretic and southern blotting methods) from those of Taylor et al. (1991), they do not change the overall relationship between haplotype 17 and the other 16 haplotypes. The mitochondrial genome of South American (BRA-90) and LIB-90 flies is almost identical (F=0.97), therefore, BRA-90 flies can tentatively be designated as haplotype 17.

We also wanted to determine whether some region of South America could be the source of the original 1988 infestation in Libya. To address this type of question, analysis of mtDNA RFLP is considered to be useful for monitoring population dispersal and maternal lineages, because the mtDNA is maternally inherited without recombi-

TABLE 4. RESTRICTION FRAGMENT PATTERN OF PCR AMPLIFIED DNA OF SCREWWORM SAMPLES.

Enzyme	Restriction Fragment Sizes Primers (refer to Table 1)		
	1	2	5
*Eco*RV			
LIB-90	1.35	.59	.85, 0.5
BRA-90	1.35	.59	.85, 0.5
OW-87	1.35	.59	.85, 0.5
COR-91	—	—	—
*Fnu*4HI			
LIB-90	1.36	.46	1.36
BRA-90	1.36	.46	1.36
OW-87	1.36	.46	1.36
COR-91	—	—	—
*Hind*III			
LIB-90	.59, .48, .22	.59	1.36
BRA-90	.59, .48, .22	.59	1.36
OW-87	.59, .48, .22	.59	1.36
COR-91	—	—	—
*Hpa*I			
LIB-90	1.35	.58	1.37
BRA-90	1.35	.58	1.37
OW-87	1.35	.58	.91, .38
COR-91	—	—	.91, .38
*Msp*I			
LIB-90	1.22	.59	1.16
BRA-90	1.22	.59	1.16
OW-87	1.22	.59	1.16
COR-91	—	—	—
*Scrf*I			
LIB-90	1.35	.59	1.22
BRA-90	1.35	.59	1.22
OW-87	1.35	.59	1.22
COR-91	—	—	—
*Ssp*I			
LIB-90	.71, .26, .07	.29, .21	1.08, .15, .08
BRA-90	.71, .26, .07	.29, .21	1.08, .15, .08
OW-87	.71, .26, .07	.29, .21	.55, .44, .15, .08
COR-91	—	—	.55, .44, .15, .08
*Sst*I			
LIB-90	1.35	.59	1.17
BRA-90	1.35	.59	1.17
OW-87	1.35	.59	1.17
COR-91	—	—	—

nation (Avise et al. 1987). Paternal contributions to the mtDNA gene pool are rare or non-existent (Lansman et al. 1983). Although several geographical samples from South America (we had access to only one strain from Brazil) should be analyzed, a high level of mitochondrial similarity (F=0.97) between Libyan and Brazilian strains indicate that some region(s) of South America could very well be the source of the 1988 infestation in Libya. Clearly, additional studies are warranted.

The second goal was to find PCR-based DNA marker to distinguish Libyan or South American flies from those of Mexico, Jamaica and Central American. As shown in Table 4, total DNA amplified by primer pair 5 (Table 1) followed by digestion with *Hpa*I or *Ssp*I produced patterns which can be used to distinguish Libyan and South American strains from those of Mexico, Jamaica and Central America.

The third goal was to determine genetic differences, if any, between the Costa Rica samples collected in 1986 and 1991. Our results (Table 3) showed that COR-91 was polymorphic for *Hind*III and *Sst*III sites, whereas COR-86 was monomorphic (data from Roehrdanz 1989). The COR-91 contained two patterns, D and G for *Hind*III and A and B for *Sst*I, whereas, COR-86 was fixed for pattern A for *Hind*III and pattern A for *Sst*I. In addition, COR-91 was fixed for pattern A for *Msp*I, whereas, COR-86 was fixed for pattern B for *Msp*I. These differences can be attributed to either livestock or human-assisted migrations of flies or simply due to sampling error.

Variation in restriction sites in mtDNA within and among populations are common (reviews by Avise & Lansman 1983, Wilson et al. 1985, Avise 1986, Avise et al. 1987, Moritz et al. 1987). Kessler & Avise (1985) reported significant spatial heterogeneity in the distribution of mtDNA variants within populations (of cotton rats). In addition, mtDNA length variation within an individual (heteroplasmy) and between individuals have been reported in other organisms (reviewed by Moritz et al. 1987). During our studies, except for one individual from COR-91, which produced a mixture of patterns D and G for *Hind*III, we did not detect any evidence of heteroplasmy in populations from South America, Libya, and a Mexican laboratory strain.

The extent of variability observed within and among populations greatly depends upon the analytical power of the method used. For example, the maternally inherited mtDNA and paternally inherited Y-linked genes generally show less variation within populations and more between populations than biparently inherited autosomal nuclear genes (Dowling & Brown 1989). Furthermore, the levels of intra- and inter-species mtDNA RFLP varies greatly among different taxa (Narang et al. 1994). Therefore, it is desirable to use both multiple techniques as well as feral samples of populations to obtain more reliable estimates of genetic variability. Our data provide useful markers to identify geographical populations of screwworm, and should not be regarded as indicators of level of genetic variability of respective geographical populations or for genetic relationships among them.

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SURVEY AND ECOLOGICAL STUDIES OF THE TERMITES (ISOPTERA: KALOTERMITIDAE) OF MONA ISLAND

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ABSTRACT

A survey of eight sites on Mona Island revealed four termite species in the family Kalotermitidae: *Incisitermes* nr. *bequaerti* (Snyder), *I.* nr. *incisus* (Silvestri), *Neotermes mona* (Banks), and *Procryptotermes corniceps* (Snyder). *Incisitermes* nr. *bequaerti* is a new record for the island. Identifiable wood hosts are reported. All species were found in dead wood, which is typical of drywood termites, but *N. mona* and *P. corniceps* were also collected from live wood. Examination of dead wood in three 9.3 m^2 plots in a dense stand of *Leucaena leucocephala* (Lam.) de Wit. revealed that an average of approximately three-quarters of the wood pieces were attacked by termites. Although the total volume of dead wood was almost six times greater in Plot 1 than in the other two plots, the number of termites per unit volume of dead wood was very similar (0.4 per cm^3). Total numbers of individuals per colony ranged from 11 to 3,359 termites. Caste composition is reported for each colony, and large variations among colonies were noted. Alates as well as eggs were more common in larger colonies. Soldier percentages ranged from 0.7% to 20.5% in 16 *P. corniceps* colonies. The largest colonies occurred when the greatest volume of dead wood was available.

Key Words: Caste, colony size, subtropical dry forest, West Indies, wood decomposition.

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RESUMEN

Un muestreo de ocho sitios en la Isla Mona reveló cuatro especies de termitas de la familia Kalotermitidae: *Incisitermes* nr. *bequaerti* (Snyder), *I.* nr. *incisus* (Silvestri), *Neotermes mona* (Banks), y *Procryptotermes corniceps* (Snyder). *Incisitermes* nr. *bequaerti* es un nuevo registro para la isla. Son reportadas las maderas hospedantes. Todas las especies fueron encontradas en madera muerta, lo cual es típico para las termitas de madera seca, pero *N. mona* y *P. corniceps* fueron también colectados en madera viva. El exámen de la madera muerta en tres parcelas de 9.3m^2 en un denso grupo de *Leucaena leucocephala* (Lam.) de Wit. reveló que un promedio de aproximadamente tres cuartos de los pedazos de madera estaban atacados por las termitas. A pesar de que el volumen total de madera muerta fué casi seis veces mayor en la parcela 1 que en las otras dos, el número total de termitas por unidad de volumen de madera muerta fue muy similar (0.4/cm^3). El número total de individuos por colonia varió de 11 a 3359 termitas. La composición de las castas es reportada para cada colonia. Fueron observadas grandes variaciones dentro de las colonias. Los porcentajes de soldados variaron de 0.7 a 20.5% en 16 colonias de *P. corniceps*. Las mayores colonias fueron encontradas cuando el mayor volumen de madera muerta era disponible.

Mona Island is a 55 km^2 limestone plateau that rises 60 to 100 m above sea level in the Mona Passage between the Caribbean islands of Puerto Rico and Hispaniola. It has no permanent settlements and, since 1973, has been under the administration of the Puerto Rican Department of Natural Resources, which has emphasized the value of Mona Island as a wilderness and research site. An overview of the history, geography, and ecology of Mona Island is provided by Cintrón (1991). The vegetation of Mona Island, which belongs within the subtropical dry forest life zone, is described in Cintrón & Rogers (1991).

A main purpose of this study was to continue a survey of the termites of Mona Island begun in the early 1990's by Jones (1991). It is the first since Ramos' (1946) extensive survey of the insects of Mona Island in 1935 and 1944. Three of the four termite species he collected were identified by A. E. Emerson as *Incisitermes snyderi* (Light), *Neotermes mona* (Banks), and *Procryptotermes corniceps* (Snyder). The identity of the fourth species was tentatively given as *I. incisus* (Silvestri), but Emerson noted that he could not be certain because he had not examined the type specimens.

Incisitermes snyderi is distributed extensively, not only in the West Indies, but also in Mexico and the southern United States (Scheffrahn et al. 1994, Snyder 1956, Weesner 1965). *Incisitermes incisus* is reported from Barbados, Dominica, Guadeloupe, Montserrat, Puerto Rico, and the Virgin Islands (British: Beef, Eustatia, Guama, Virgin Gorda; U.S.: St Croix) (Snyder 1956, Scheffrahn et al. 1994).

Procryptotermes corniceps, the only Neotropical representative of this genus, is reported from many islands in the West Indies (Scheffrahn et al. 1994). It is listed as the most common kalotermitid in natural vegetation on Providenciales and Grand Turk Islands in the British West Indies (Scheffrahn et al. 1990). The original description of *N. mona* was by Banks (1919) from soldiers collected on Mona Island, which remained for several decades the species' only reported locality (Ramos 1946, Snyder 1956). *Neotermes mona* is now known to be common on Providenciales and Grand Turk Islands (Scheffrahn et al. 1990), and it also has been reported from the Dominican Republic (Hispaniola) and Guana (British Virgin Islands) (Scheffrahn et al. 1994).

The four termite species found on Mona Island are drywood termites belonging to the primitive family Kalotermitidae. Colonies live within their food sources of dry

wood, chiefly dead branches and tree trunks. They do not require contact with the soil for moisture. Rather, the termites obtain water as a metabolic by-product of cellular metabolism and also from external water and, possibly, living plant tissue. Colonies consist of a pair of primary reproductives (king and queen) or replacement reproductives, soldiers, nymphs, pseudergates, larvae, and eggs. Winged imagoes (alates) may be produced and, if so, they swarm seasonally to establish new colonies. Mature drywood colonies generally do not exceed a few thousand individuals (Nutting 1969, Lenz 1994). Because of their habit of living within dry wood and their relatively small colony sizes, it is possible to collect entire colonies. This made it feasible to collect entire colonies on which to base the other major purposes of this study, which were to determine: kalotermitid colony size, caste composition, and the relationship between the number of termites and available dead wood volume.

MATERIALS AND METHODS

Survey

Our survey of the termites of Mona Island was conducted from March 24 through 30, 1993. We intensively sampled in the vicinity of eight collection sites including the airstrip, Carabinero Beach, El Faro, Pájaros Beach, Sardinera Beach, Uvero Beach, Vereda del Centro, and Vereda India (Fig. 1). These sites were selected because of ease of access via roads or trails.

Termites were removed from standing or fallen dead wood using hatchets, wood chisels, saws, and forceps. Groups of termites with representative castes were placed in vials containing 85% ethanol. We collected soldiers and pseudergates, and alates if available. The host plant was identified whenever possible using keys and figures in Little & Wadsworth (1964) and Little et al. (1974); we also relied on local expertise.

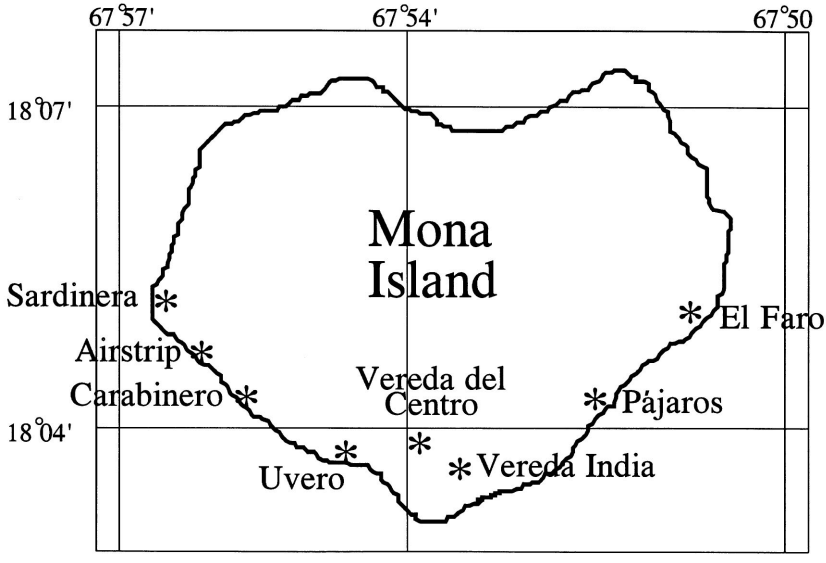

Fig. 1. March 1993 termite collection sites (*) on Mona Island.

Termites were identified to species with the aid of taxonomic keys (Banks 1919, Snyder 1956). A majority of determinations were verified by R. H. Scheffrahn (University of Florida) and J. Krecek (University of Florida) and included comparisons with reference specimens. Voucher specimens have been deposited in laboratory collections of the senior author, as well as with the International Institute of Tropical Forestry in Río Piedras, Puerto Rico and with the Puerto Rican Department of Natural Resources museum on Mona Island.

Volume of Available Dead Wood, Colony Density, and Caste Composition

A site was selected SSW of the airstrip in a large stand of leadtrees [*Leucaena leucocephala* (Lam.) de Wit.]. Live trees in the stand averaged 2.7 cm diam breast height (n = 32) (Fig. 2). Three points were randomly selected, then used as centers for three 3.05×3.05 m plots with borders aligned along compass points. All dead wood ≥1.2 cm in diam was collected (standing dead trunks, branches or trunks on the ground, dead branches on live trees, and dead branches detached but suspended in undergrowth), except for two dead branches that could not be reached in the crowns of trees in Plot 1 and thus were excluded from all calculations. The length (longest continuous dimension) and midpoint of branches were measured and used to calculate wood volume ($\pi r^2 L$). Standing dead trunks were measured at breast height. Because live wood was extremely dense and difficult to split, it generally was not feasible to determine live wood volume when termite galleries extended into living portions of trees.

Each piece of dead wood was split open and examined for any signs of termite activity. Termite galleries, termite fecal pellets, and/or body parts were noted as evidence of former termite activity. When live termites were observed, every attempt was made to collect the entire colony into 85% ethanol. Each entire colony collection was

Fig. 2. A dense stand of *Leucaena leucocephala* along the airstrip on Mona Island.

later examined in the laboratory using dissecting microscopes. All termites were sorted by categories: 1) primary reproductives, 2) alates and de-alates, 3) soldiers, 4) pseudergates and nymphs with short wing pads (tip of mesothoracic wing pad not extending beyond thoracic segments), 5) nymphs with long wing pads, and 6) larvae (first three instars). We were unable to differentiate lightly pigmented replacement (neotenic) reproductives. Exact counts of each category were made. The presence of eggs was noted.

Results and Discussion

Survey

Seventy-seven termite samples were collected during the survey. Members of the family Kalotermitidae were the only termites collected on Mona Island. Four species were identified: *I.* nr. *bequaerti* (Snyder), *I.* nr. *incisus*, *N. mona*, and *P. corniceps*. At least one collection of each species included alates. *Incisitermes* nr. *bequaerti* represents a new record for Mona Island. The other species collections corroborate previous records for the island (Banks 1919, Ramos 1946, Jones 1991). However, termites reported as *I.* nr. *snyderi* by Jones (1991) should have been classified as *I.* nr. *incisus*.

Procryptotermes corniceps was the most common and widely distributed species; it was found at every site sampled. Our observations suggest that *P. corniceps* more readily tolerates hot, dry conditions than the other termites on Mona Island. Among the hosts of *P. corniceps* were dead *Coccoloba uvifera* (L.) L., *Gossypium barbadense* L., *Hippomane mancinella* L., *Tamarindus indica* L., dead and live wood of *L. leucocephala*, and others that were unidentifiable.

Our collections suggest that *N. mona* is neither widely nor commonly distributed and occurs primarily along the west coast of Mona Island. Of the sites sampled, this species was found only in the Sardinera Beach area and the airstrip (Fig. 1). *Neotermes mona* was previously collected at Uvero Beach (Ramos 1946, Jones 1991) and the airstrip (Jones 1991). We recovered *N. mona* from dead and live wood of *L. leucocephala* and from dead wood of *Bursera simaruba* (L.) Sarg. We also identified this termite species from a sample collected by G. Hernández (P.R. Dept. Natural Resources) during April 1993 in *Melicoccus bijugatus* Jacq. at Sardinera Beach.

Incisitermes nr. *incisus* was found at all eight sites, and was particularly common at El Faro. Many colonies included two soldier morphs: long- and short-headed forms. This termite was found in *H. mancinella*, *L. leucocephala*, and dead wood that was unidentifiable.

Incisitermes nr. *bequaerti* was collected at Carabinero Beach, Uvero Beach, Vereda del Centro, and Vereda India. It was collected from *C. uvifera* and dead wood that was unidentifiable to species.

Volume of Available Dead Wood, Colony Density, and Caste Composition

The total volume of available dead wood in the three plots was 20,671 cm^3; 3,242 cm^3; and 3,153 cm^3, respectively. A large percentage of the wood showed current or past signs of termite occupation: Plot 1, 74.3%, n = 35; Plot 2, 87.5%, n = 24; Plot 3, 66.7%, n = 21. Overall, slightly more than three-quarters of the available pieces of dead wood showed evidence of termites. Of these, however, current termite infestations were less common than colonies that had met their demise (Plot 1, 26.9% live; Plot 2, 28.6% live; Plot 3, 42.9% live).

TABLE 1. MONA ISLAND DRYWOOD TERMITE COLONY COMPOSITION IN DEAD WOOD.

Species[1]	Sample #	Wood Volume (cm^3)	Larvae	Pseudergates & Short Wing-pad Nymphs	Long Wing-pad Nymphs	Soldiers	Primary Reproductives	Eggs[2]	Alates	Total
PLOT 1										
Pc	113	1,079.9	0	24	44	3 (4.2%)	0	0	0	71
Pc	132	3,315.8	18	117	98	7 (2.8%)	0	0	13 (5.1%)	253
Pc	114	862.1	36	298	3	26 (7.1%)	2	+	0	365
Pc	118	3,541.9[3]	21	801	91	64 (6.4%)	0	+	15 (1.5%)	992
Pc	117	1,012.8 3,607.0[3]	102	1,574	189	76 (3.8%)	2	+	50 (2.5%)	1,993
Pc	131	2,491.4[4]	123	2,322	439	117 (3.8%)	2	+	81 (2.6%)	3,085[5]
Pc	116	7,458.2	216	2,346	526	173 (5.2%)	2	+	96 (2.9%)	3,359
Undet.	115	112.3	3	31	23	0 (0.0%)	0	0	0	57
PLOT 2										
Pc	168	40.8[4]	0	8	1	2 (18.2%)	0	0	0	11
Pc	164	133.6	0	8	0	2 (16.7%)	2	0	0	12
Pc	163	274.1	13	58	0	1 (1.4%)	0	+	0	72
Pc	166	53.8	9	62	0	18 (20.2%)	0	+	0	89
Pc	165	59.9	5	78	31	1 (0.7%)	0	+	11 (8.7%)	126
Pc	167	345.4[4]	39	784	254	17 (1.5%)	2	+	51 (4.4%)	1,147
PLOT 3										
Pc	172	128.7[4]	0	189	2	22 (10.3%)	0	0	0	213
Pc	173	370.0[4]	11	143	16	50 (20.5%)	0	+	24 (9.8%)	244
Pc	174	386.2[4]	31	468	1	77 (13.3%)	2	+	0	579
Ii	171	19.3	0	8	0	5 (38.5%)	0	0	0	13
Ii	169	77.4	31	178	0	23 (9.8%)	2	+	0	234
Undet.	170	76.3	0	46	0	0 (0.0%)	0	0	0	46

[1]Pc = Procryptotermes corniceps; Ii = Incisitermes nr. incisus; Undet. = Undetermined.
[2]Presence (+) or absence of eggs noted.
[3]Total volume of live wood containing termite galleries.
[4]Live wood volume that contained termite galleries was not assessed.
[5]Includes one damaged and unidentifiable.

Procryptotermes corniceps and *I.* nr. *incisus* were the only species found in the sample plots, although *N. mona* was found outside the plots in the same stand of leadtrees. *Procryptotermes corniceps* was by far the predominant termite.

In some cases (n = 8) of dead branches on live trees, termite galleries extended into the living portion of the tree (Table 1). Because this live wood either was not sampled or was incompletely sampled, totals for these colonies are underestimated. However, our observations of *N. mona* and *P. corniceps* in live wood indicated that colonies probably were initiated in an attached dead branch, then extended into the living portion of the tree as the colony grew. Whereas dead wood was extensively riddled with galleries, often just a single termite gallery penetrated into the core of the live wood. Drywood termite species that can extend foraging galleries from the originally colonized dead wood into regions of live wood in a tree attain larger colony sizes than species that remain solely in the dead wood that initially was invaded (Lenz 1994). Maximum colony size of *P. corniceps* greatly exceeded that of *I.* nr. *incisus*, although the latter species was much less common in the plots (Table 1).

Total termite counts and caste composition for the 20 colonies found in the 3 plots are reported in Table 1. These data, which indicated that colony size of these two drywood termite species did not exceed 3,500 individuals, are consistent with previous reports for the Kalotermitidae (Nutting 1969, Lenz 1994).

Although the total volume of dead wood was approximately six times greater in Plot 1 than in the other two plots, the number of termites per unit volume of dead wood was very similar: 0.37 termites per cm^3 in Plot 1, 0.45 termites per cm^3 in Plot 2, and 0.42 termites per cm^3 in Plot 3. The similarity of these data likely stems from the fact that average colony size coupled with average available wood volume per colony was greater in Plot 1 than in the other two plots. In Plot 1, colonies averaged 1,198 termites, and dead wood volume per colony averaged 2,553 cm^3; in Plot 2, colonies averaged 243 termites, and wood volume averaged 151 cm^3; in Plot 3, colonies averaged 222 termites, and wood volume averaged 176 cm^3. These calculations exclude colonies 117 and 118 in Plot 1, because some of these termites were extracted from live wood.

Linear regression indicates a positive relationship between the $\log_{10}$ of dead wood volume and the $\log_{10}$ of colony size ($y = 0.783x + 0.351$) (Fig. 3). With an R^2 of 0.501, linear regression explains more than half of the variation in the data. Thus, drywood termite colonies on Mona Island attain larger sizes in larger pieces of dead wood. The size of mature drywood termite colonies typically is positively correlated with food resource size (Lenz 1994).

Colony density ranged from 0.6 colonies per m^2 in Plots 2 and 3, to 1.2 colonies per m^2 in Plot 1. These high densities probably are representative of the termite population in the *Leucaena* stand but not of Mona Island as a whole. Observations during our survey, however, indicate a relatively high density of drywood termite colonies in other habitats on the island.

Termite densities in the dry forests of Mona Island were severalfold higher than those observed in *Cyrilla racemiflora* L. in the Luquillo tropical montane forest of Puerto Rico (Torres 1994). This tends to support the hypothesis that termites are more important as wood decomposers in dry forests, whereas fungi are more important in wet forests (Chudnoff & Goytía 1972, Bultman & Southwell 1976). The dry forest life zone is a significant component in the tropics and subtropics, comprising approximately 42% of forest lands (Murphy & Lugo 1986).

Caste composition varied with colony size (Table 1). All colonies with ≥992 individuals (n = 5) contained eggs as well as alates. Small colonies with both these developmental stages could be found, but not consistently; alates and eggs were found in 2 small colonies, one with n = 126 termites and the other with n = 244 termites. The

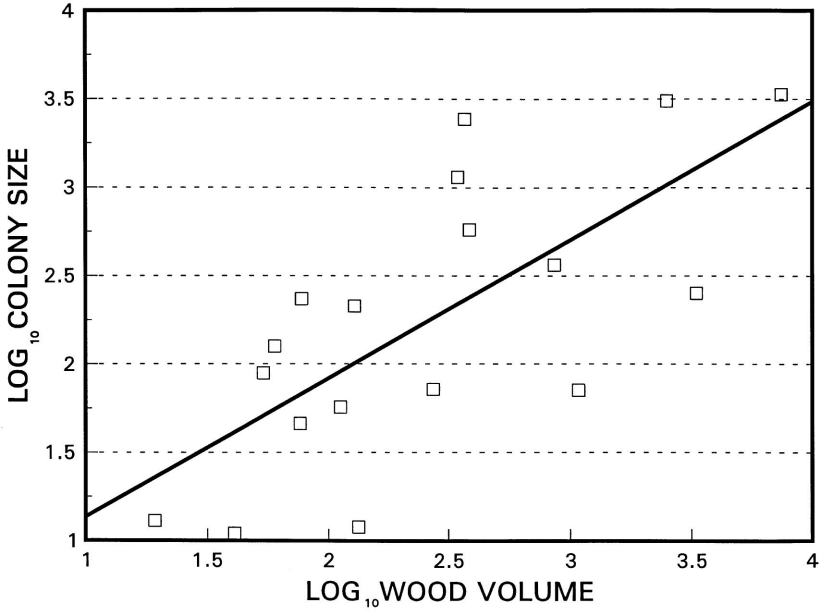

Fig. 3. Relationship between the $\log_{10}$ of dead wood volume and the $\log_{10}$ of drywood termite colony size on Mona Island ($y = 0.783x + 0.351$; $R^2 = 0.501$), ($n = 18$).

smallest colony with alates present was comprised of 126 termites. The 6 smallest colonies (≤71 termites) had neither eggs nor alates and may represent incipient colonies or those on the decline. We suspect that these colonies were at the end of their colony cycle because of the absence of primary reproductives and early instars. Only one of the six smallest colonies contained primary reproductives.

Soldier percentages ranged from 0.7% to 20.5% for the 17 *P. corniceps* colonies and were 9.8% and 38.5% for the two colonies of *I.* nr. *incisus*. Soldier percentages were more variable for small colonies than for large colonies.

In conclusion, this survey revealed four species of kalotermitids, including *I.* nr. *bequaerti*, which is a new record for Mona Island. These termites were found in a variety of tree species. Drywood termites apparently are important wood decomposers in the subtropical dry forest life zone of Mona Island, with an average of approximately three-quarters of dead wood branches showing signs of termite attack. Approximately 0.4 termites per cm^3 of dead wood were noted. Colony size of *P. corniceps* ranged from 11 to 3,359 individuals, with large variations in caste composition among colonies. Data on wood volume together with total termite counts support the hypothesis that kalotermitid colony size is closely attuned to the size of the food resource.

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THE FIRST OCCURRENCE OF *OZOPHORA HEYDONI* IN FLORIDA WITH THE DESCRIPTION OF A NEW SPECIES OF *OZOPHORA* FROM THE NEOTROPICS (LYGAEIDAE: HEMIPTERA)

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ABSTRACT

The discovery of a species of *Ozophora* not previously reported for Florida led to a re-examination of the *Ozophora atropicta* complex. This examination showed that *O. heydoni* had been incorrectly synonymized and is resurrected from synonymy and reported from Florida for the first time. A new species, *Ozophora atropictoides*, is described from Trinidad.

Key Words: *Ozophora*, Florida, synonymy, *atropictoides*.

RESUMEN

El descubrimiento de una especie de *Ozophora* no previamente reportada de la Florida condujo a un reexamen del complejo de *Ozophora atropicta*. Este examen mostró que *O. heydoni* habia sido incorrectamente sinonimizada y es resucitada de la sinonimia y reportada de la Florida por primera vez. Una nueva especie, *Ozophora atropictoides*, es descrita de Trinidad.

The discovery of a species of *Ozophora* not previously reported for Florida led to re-examination of the *Ozophora atropicta* complex. Recently the junior author and Mrs. Holly Glenn, Biological Scientist II, Tropical Research and Education Center, collected a number of specimens of a species of *Ozophora* on the grounds of the Tropical Research and Education Center (TREC) under a large *Ficus* tree. This *Ozophora* was determined to be a species not known from Florida, either *O. atropicta* or a closely related species. A comparison of the male genital capsule, parameres and cuplike sclerite (Schaefer, 1977) of *O. atropicta* from the Dominican Republic with those of specimens collected at TREC showed distinct differences.

Slater & Hassey (1981) discussed the status of *O. atropicta* Barber in detail. They pointed out that the type series was mixed. Material not conspecific with the holotype was subsequently described as *O. levis* by Slater & Baranowski (1983). Slater & Hassey (ibid) also synonymized *O. heydoni* Barber & Ashlock with *O. atropicta*. They discussed variation in, and distribution of *O. atropicta*, hypothesizing the origin and dispersal of West Indian populations from a mainland source area.

It is now apparent that Slater & Hassey (1981) were incorrect in synonymizing *O. heydoni* and *O. atropicta* and in believing that the genitalia do not differ significantly. These taxa, which we now believe is a complex of species, are very similar externally, differing externally chiefly by characters discussed by Slater & Hassey in their discussion of "variation" between Bahamian and Greater Antillean populations. However, we now find the shape of the genital capsule to be consistently different. This structure has proven to be diagnostic for many closely related species of *Ozophora*. Furthermore, mainland populations of what Slater & Hassey believed to be *O. atropicta* also have a distinctly different genital capsule.

Three distinct species are thus involved:

1. *Ozophora heydoni* Barber & Ashlock NEW STATUS, occurs in extreme southern Florida (Homestead, Fla. I-31-1991 R. M. Baranowski, light trap and under *Ficus*) and probably throughout the Bahamas. The holotype is from New Providence, paratypes from Abaco Cays, Great Abaco I. and Andros I. Slater & Hassey (1981) also report it (as *O. atropicta*) from Eleuthera Is. (Current Cut, Powell Pt.), Arthur's Town Cat Is.

In *O. heydoni* the posterior margin of the genital capsule has a slight mesal protrusion when viewed dorsally (Fig. 3) and in lateral view this protrusion can be seen as a caudal projection of the capsule (Fig. 2). The arms of the cuplike sclerite are widely separated and evenly conical with heavily sclerotized distal ends (Fig. 1).

The paramere of *O. heydoni* (Fig. 11) has a large toothlike inner projection that extends over the base of the outer projection. There is no minute secondary tooth.

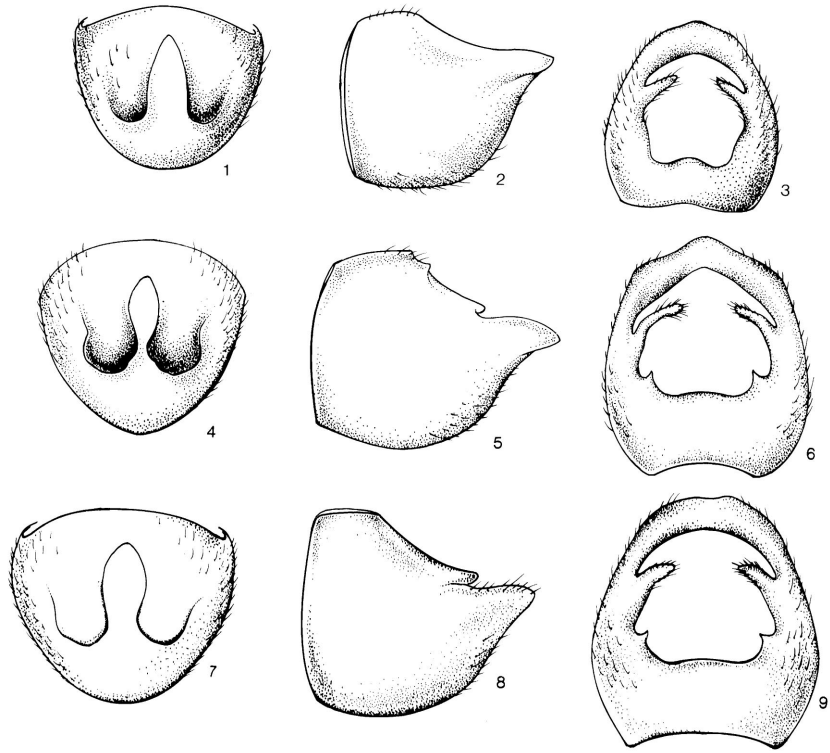
Ozophora heydoni keys to couplet four in Slater & Baranowski (1983, 1990). It can be separated from *O. levis* by the pale apex of the membrane, and the variegated hemelytra. In some specimens, the pale membrane apex is obscure. In such cases, *O. heydoni* may be distinguished from *O. levis* by having the lateral corial margins entirely pale and in contact with the pale subapical macula, whereas in *O. heydoni* the lateral corial margins are separated from the subapical pale macula by a large dark transverse band. In *O. levis,* the posterior pronotal lobe is, for the most part, concolorous with the anterior lobe, whereas in *O. heydoni* the posterior lobe is sometimes pale and contrasts markedly with the dark anterior lobe. Such specimens of *O. heydoni* key to couplet seven where they can be separated from *O. caroli* by lacking a yellow scalloped posterior pronotal margin and from *O. floridana* by having one or more dark bars on the clavus and anterior one-half of the corium.

2. *Ozophora atropicta* Barber as here restricted appears to be confined to the Greater Antilles. Slater & Hassey (1981) reported numerous records from Cuba (misspelled as Cubra in text) and the Dominican Republic. The holotype is from Puerto Rico.

In nominal *O. atropicta*, the posterior margin of the genital capsule is conspicuously produced (Fig. 6), resulting from a strong, almost fingerlike projection backward of the posterior margin of the capsule (Fig. 5). The arms of the cuplike sclerite are divergent, but almost in contact along the midline at their inner angles and are strongly bent and heavily sclerotized distally (Fig. 4).

The paramere (Fig. 10) has a much smaller, less toothlike inner projection that does not extend over the base of the outer projection, and also has a minute secondary tooth.

3. *Ozophora atropictoides* **New Species**. The restriction of *O. atropicta* to the West Indies and *O. heydoni* to the Bahamas and extreme southern Florida leaves mainland populations that have previously been referred to *O. atropicta* without a name; these are described below as a new species. We have examined the genital capsules of spec-



Figures 1-9. Male genital capsule showing cup-like sclerite. Posterior view. Fig. 1 *O. heydoni*; Fig. 4 *O. atropicta*; Fig. 7 *O. atropictoides*; Male genital capsule lateral view. Fig. 2 *O. heydoni*; Fig. 5 *O. atropicta*; Fig. 8 *O. atropictoides*; Male genital capsule dorsal view. Fig. 3 *O. heydoni*; Fig. 6 *O. atropicta*; Fig. 9 *O. atropictoides*.

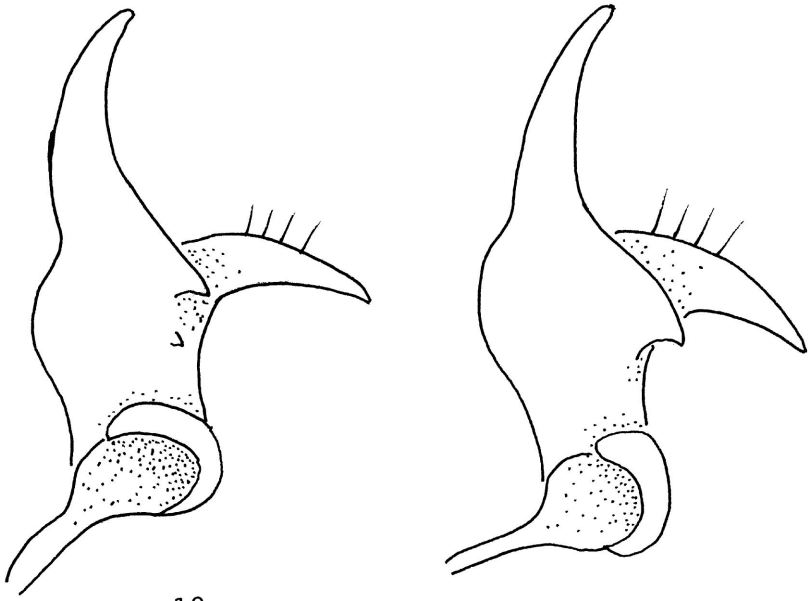
imens from Mexico, Central America, and Trinidad and all show characteristics described below.

In *O. atropictoides*, the posterior margin of the genital capsule lacks a caudal projection (Figs. 7, 9) and, in lateral view, the capsule can be seen to slope evenly posteriorly from the ventral to dorsal margins and thus has a less noticeable backward protrusion dorsally (Fig. 8). The arms of the cuplike sclerite are strongly divergent and not heavily sclerotized at their distal ends (Fig. 7).

Slater & Hassey's (1981) discussion of the dispersal vs. vicariance hypothesis for the populations of these species still has relevance despite their mistaken view that a single species was involved. It suggests, however, that a longer time period was involved and makes the absence of the complex from Jamaica less puzzling.

Ozophora atropictoides Slater & Baranowski, **New Species**

Head and anterior pronotal lobe dark red-brown. Anterior and lateral pronotal margins and entire posterior pronotal lobe strongly and contrastingly pale yellow. Scutellum chocolate brown with two divergent yellow vittae on distal one-third, these not attaining base of scutellum. Hemelytra chiefly pale yellow, with large dark macula present laterad of radial vein with anterior margin at level of distal end of claval



Figures 10-11. Parameres lateral view: Fig. 10 *O. atropicta*: Fig. 11 *O. heydoni*.

commissure, second large macula at apex of corium. Membrane fumose. Legs, first and second antennal segments pale yellow. Third antennal segment also yellow, but darkened near distal end. Fourth segment fuscous with conspicuous large white subbasal annulus on proximal third. Punctures dark brown, small and well separated from one another. Body nearly glabrous above (few very short inconspicuous hairs present when viewed laterally).

Head slightly declivent anteriorly, reaching over basal one-third of first antennal segment. Eyes very large, sessile, occupying most of lateral head surface. Length head 0.90, width 1.00, interocular space 0.30. Lateral pronotal margins carinate; calli granulose, well separated from one another mesally, very sparsely punctate. Length pronotum 1.02, width 1.56. Length scutellum 1.04, width 0.88. Lateral corial margins evenly but shallowly concave. Length claval commissure 0.80. Midline distance apex clavus-apex corium 1.32. Midline distance apex corium-apex abdomen 0.92. Metathoracic scent gland auricle short, bent slightly caudolaterally; evaporative area occupying inner 2/3 of anterior lobe of metapleuron, outer margin straight. Forefemora moderately incrassate, armed below with three major spines followed proximally by 4-5 hair spines. Labium extending posteriorly well between metacoxae, first segment attaining or slightly exceeding base of head. Length labial segments I 0.90, II 0.94, III 0.64, IV 0.40. Antennae elongate, terete, fourth segment narrowly fusiform. Length antennal segments I 0.66, II 1.80, III 1.40, IV 1.52. Total body length 5.88.

Male genital capsule as in figures 7-9.

TYPES. Holotype. Male. TRINIDAD: Simla, Arima-Blanchisseuse Rd. 600 ft. VII-20-1975 (J. Price) (blacklight trap). In American Museum of Natural History. Paratypes: TRINIDAD: 15 males, 15 females same data as holotype. 1 male same ex-

cept VII-14-1975. 1 female Simla Arima Valley II-4-1965 (J. A. Slater & N. T. Davis. 1 male, 1 female St. Augustine VI-14-1973 (R. Baranowski, F. O'Rourke, V. Picchi, J. Slater) (light trap). In National Museum of Natural History (USNM), R. M. Baranowski and J. A. Slater collections.

Although the posterior lobe of the pronotum in the holotype is entirely pale, and the hemelytra chiefly so, this is not true of most of the type series. This species appears to be sexually dimorphic. Females, in addition to being larger, are usually very dark with the hemelytra predominately dark chocolate brown. Many males also are much darker than the holotype. Specimens of both sexes usually have a dark stripe on the meson of the posterior pronotal lobe. They frequently have additional dark striping on the posterior pronotal lobe and have the anterior third of the corium with a dark macula. The darker coloration is found in most Central American specimens as well.

Specimens listed by Slater & Hassey (1981) from Mexico, Honduras, Costa Rica, Panama and Venezuela have been reexamined and appear to be *O. atropictoides*. The Brazilian material listed by Slater & Hassey also appears to represent this species with the exception of the male and female from "Corupa (Hans Humbolt) S. Cat. XI-1944" which we believe represents neither *O. atropictoides* nor *O. atropicta*.

ETYMOLOGY. Referring to a similarity to *O. atropicta*.

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THERMOREGULATION BY ENDOGENOUS HEAT PRODUCTION IN TWO SOUTH AMERICAN GRASS DWELLING CICADAS (HOMOPTERA: CICADIDAE: *PROARNA*)

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ABSTRACT

Proarna bergi (Distant) and *Proarna insignis* Distant use metabolic heat to raise body temperature (T_b) for activity when ambient conditions would prevent activity in ectothermic animals. Both species were observed singing during overcast or rainy conditions and at dusk. T_bs in the field exceeded ambient by as much as 7.4°C when solar radiation was unavailable to the insects. In the laboratory voluntary metabolic heat production raised T_b as much as 12.3°C and 10.7°C above ambient in *P. bergi* and *P. insignis* respectively. Estimates of metabolic rate from heating and cooling curves were 0.118 ml O_2 per min for *P. bergi* and 0.126 ml O_2 per min for *P. insignis*. Fine shiver-like movements of the thoracic musculature produced the heat. The T_b at which endogenous warm-up voluntarily stopped in the laboratory was similar to the T_bs measured in active animals in the field. Thermal responses measured in the laboratory also illustrate these animals are thermoregulating with endogenous heat. Endogenous heat production uncoupled reproductive behavior from environmental constraints.

Key Words: Endothermy, thermoregulation, temperature, shivering, cicadas, Cicadidae, *Proarna*

RESUMEN

Proarna bergi (Distant) y *Proarna insignis* Distant utilizan calor metabólico para elevar la temperatura del cuerpo (T_b) y ser activos cuando las condiciones ambientales impiden la actividad en otros animales ectotérmicos. Ambas especies fueron observadas cantando bajo condiciones de cielo completamente nublado, bajo la lluvia y en la obscuridad. Las T_b en el campo excedieron las del ambiente en 7.4°C cuando la radiación solar no era disponible a los insectos. En el laboratorio la producción voluntaria de calor aumentó la T_b en 12.3°C y 10.7°C por encima del ambiente en *P. bergi* y *P. insignis* respectivamente. Los estimados de la tasa metabólica de las curvas de calentamiento y enfriamiento fueron de 0.118 ml de O_2 por minuto para *P. bergi* y 0.126 ml de O_2 por minuto para *P. insignis*. Finos movimientos en forma de temblor de la musculatura torácica producen el calor. La T_b a la cual el calentamiento endógeno voluntariamente cesó en el laboratorio fué similar a la T_b medida en animales activos en el campo. Las respuestas térmicas medidas en el laboratorio además ilustran que estos animales son termorreguladores con calor endógeno. La producción de calor endógeno

posibilitó el desarrollo de la conducta reproductiva independientemente de las condiciones ambientales.

Cicadas must maintain their body temperature within a small range to coordinate reproductive activity (Heath 1967; Heath 1972). Thermoregulation is generally accomplished by altering the uptake of solar radiation (Heath 1967; Heath & Wilkin 1970; Heath et al. 1972; Sanborn et al. 1992). Endothermy was first described in cicadas by Bartholomew & Barnhart (1984) and has recently been described as a mechanism of thermoregulation in cicadas (Sanborn et al., in press).

This paper describes and quantifies an additional mechanism of endogenous heat production and its utilization by two grass dwelling cicadas in northern Argentina.

MATERIALS AND METHODS

Insects

Proarna bergi (Distant) and *Proarna insignis* Distant are medium-sized cicadas. Live weight averages 342 ± 12 (n=41) and 434 ± 14 (n=21) milligrams respectively. Although the data was collected mainly from males, both sexes were used in field and laboratory measurements. Animals captured for laboratory experimentation were placed in a cardboard container along with plant samples and a wet paper towel. The containers were kept on ice until the experiments could be performed during the afternoon or evening of the day of capture. Live weights were measured on a Cent-O-Gram triple beam balance (Model CG 311, Ohaus Scale Corporation) accurate to ± 5 mg. All statistics are presented as mean ± standard error. Voucher specimens are deposited in the collection of the Museo Nacional de La Plata, La Plata, Argentina.

Temperature Measurements

Equipment. Body temperature (T_b) of the cicadas was measured with a Sensortek Model BAT-12 digital thermocouple thermometer with a type MT-29/1 copper/constantan hypodermic microprobe (accurate to ± 0.1°C) which had been calibrated with a National Bureau of Standards mercury thermometer. The probes were inserted dorsally midway into the mesothorax of each cicada to measure T_b. All T_bs were measured within five seconds of the insect being captured or the insect performing the behavior under study.

Laboratory Measurements. Laboratory experiments were performed to determine the range of temperature in which the cicadas can be fully active. The procedures used to determine the thermal responses were described in previous cicada studies (Heath 1967; Heath & Wilkin 1970).

Cooling curves of *P. bergi* and *P. insignis* were measured from tethered cicadas. A copper/constantan thermocouple wire (30 gauge) was implanted into and secured to the dorsal mesothorax of the cicadas to measure changes in T_b. The tethered animal was permitted to fly to increase T_b above ambient. The animal was placed into a styrofoam box immediately after the flight terminated. The box served as a controlled radiant environment and prevented forced convective heat loss. T_b was recorded with the BAT-12 every 15 sec as the animal cooled until T_b remained relatively constant (10 min).

Heat production was determined by inducing warm-up behavior in room temperature animals (T_a 21-27°C) and measuring the duration of warm-up behavior and the difference between T_b and T_a when the animal stopped warm-up behavior. This provided a rate of warm-up for the calculation of heat production. Warm-up behavior was induced by gently tapping the dorsal thorax of a cicada with a finger. The mechanical disturbance was usually sufficient to induce endogenous heat production. T_bs at the end of warm-up were measured with the BAT-12 system. Specimens were secured by the wings before probe insertion to prevent conductive heat transfer between our fingers and the insect.

Oxygen consumption (V_{O_2}) was estimated using the following formula (Heath & Adams 1969):

$$V_{O_2} = \frac{(\Delta T \text{ ¥ sp. ht. ¥ mass})}{O_2 \text{ cal. eq.}} + \frac{(Q_l)(T_{th}-T_a)}{O_2 \text{ cal. eq.}}$$

where ΔT = rate of change of temperature during heating (°C/min), sp. ht. = specific heat of the object [.83 cal/g°C (Bartholomew 1981)], mass = thoracic mass in grams, O_2 cal. eq. = the caloric equivalent of oxygen (4.8 cal/ml O_2), Q_l = heat loss to the environment (cal/min°C), and (T_{th}-T_a) = thoracic temperature minus ambient temperature (°C). The thoracic mass of the individual *P. bergi* and *P. insignis* used to produce our cooling curves was 32.4% and 36.8% of total body mass, respectively. We used these percentages for determining thoracic mass in all calculations. We calculated V_{O_2} from the rate of heat change so direct comparisons could be made with published data.

Field Temperatures. The T_bs of singing cicadas were measured in the field in an attempt to ascertain the T_b range in which the cicadas are normally active. Insects in the field were caught either with an insect net or by hand. After an insect was captured in a net, the net was constricted around the animal to prevent movement. The thermocouple was inserted through the net into the insect to obtain the T_b measurement within five seconds of capture. This procedure prevented heat transfer between the insect and experimentor while the specimen was being oriented for insertion of the temperature probe. The insect was shaded by the experimentor during the measurement to prevent solar heating.

It was preferable to catch the insects in a net but the habitat sometimes made capturing a cicada with a net impossible. If a cicada had to be captured by hand, it was oriented immediately after capture so that it could be held by the wingtips between the thumb and forefinger. This method of restraint reduces conductive heat transfer during the capture before the T_b is recorded and minimizes change in T_b due to heat exchange with the experimentor. Ambient temperature (T_a) was recorded in the shade at a height of approximately one m above ground.

Results

Thermal responses of the two *Proarna* species are summarized in Table 1. Mean values for all thermal parameters are lower in *P. insignis* than in *P. bergi*. Minimum flight temperature (MFT) ($t=1.84$, d.f.=50, $p<.04$) and heat torpor temperatures (HT) ($t=4.90$, d.f.=55, $p<<.001$) are significantly lower in *P. insignis*, but there is no significant difference in maximum voluntary tolerance temperature (MVT) ($t=1.63$, d.f.=55, $p=.0545$). The MVT represents an upper thermoregulatory temperature (Heath 1970).

Endogenous heat to raise T_b in cicadas is produced by the thoracic musculature. *P. bergi* and *P. insignis* raise T_b by producing fine shiver-like movements of the wings. The wings of a cicada at rest are held in an “umbrella” position over the body. When

TABLE 1. TEMPERATURE RESPONSES (°C, $\overline{X} \pm$ S.E.) OF ARGENTINE ENDOTHERMIC CICADAS.

Species	Minimum Flight Temperature	Maximum Voluntary Tolerance	Heat Torpor
Proarna bergi	20.7 ± 0.51	37.6 ± 0.38	46.3 ± 0.26
	(n=34)	(n=40)	(n=39)
Proarna insignis	19.3 ± 0.40	36.4 ± 0.60	44.0 ± 0.37
	(n=18)	(n=17)	(n=18)

endogenous warm-up is initiated in the two *Proarna* species, the costal margin of the wings is raised laterally, flattening the wings in the dorso-ventral plane. The wings are then vibrated in a shiver-like motion of the thoracic musculature to produce heat.

Rates of cooling and estimated V_{O_2} determined from the heating and cooling curves for the species in this study are shown in Table 2. The rate at which cicadas cool is consistent with Newton's Law of cooling (r^2>.99 when an exponential model was fit to the data) so the animals were not regulating heat loss.

Maximum T_b differences upon cessation of warm-up behavior measured in the laboratory in *P. bergi* and *P. insignis* are 12.3°C and 10.7°C greater than T_a, respectively. The histogram of the body temperature of cessation of warm-up behavior shows a bimodal distribution (Figs. 1 and 2). This distribution may represent the T_b range of reproductive activity for the particular species and suggests that the cicadas are regulating heat production to achieve a certain body temperature. Mean T_b when warm-up behavior stopped was 33.60 ± 3.70°C (n=14) for *P. insignis* and 33.81 ± 3.04°C (n=23) for *P. bergi*. There is no significant difference in the T_b of cessation of warm-up behavior between the two species (t=0.187, d.f.=35, p=.43).

Figure 2 also shows the relationship between T_b of *P. insignis* active in the field (singing temperatures) and the T_b distribution of the species when it ceases voluntary warm-up behavior (field temperatures were measured under an overcast sky with T_a = 31.9°C). The upper peak of the warm-up temperature distribution is greater than or equal to the modal singing temperature in *P. insignis*. The figure demonstrates that the cicadas possess the mechanisms necessary to raise T_b endothermically to the level necessary for activity and that heat production is regulated to maintain T_b in a biologically significant temperature range. The upper peak of the bimodal distribution for both species encompasses the MVT temperatures determined for each species.

TABLE 2. COOLING RATES AND ESTIMATED OXYGEN CONSUMPTION (V_{O_2}) OF THE CICADAS DETERMINED IN THE PRESENT STUDY. REPORTED VALUES ARE MEAN ± STANDARD ERROR.

Species	Cooling Rate (°C/min.°C)	V_{O_2} Max (ml O_2/min)
Proarna bergi	0.282	0.110 ± 0.0176
	(n=1)	(n=6)
Proarna insignis	0.257	0.132
	(n=1)	(n=1)

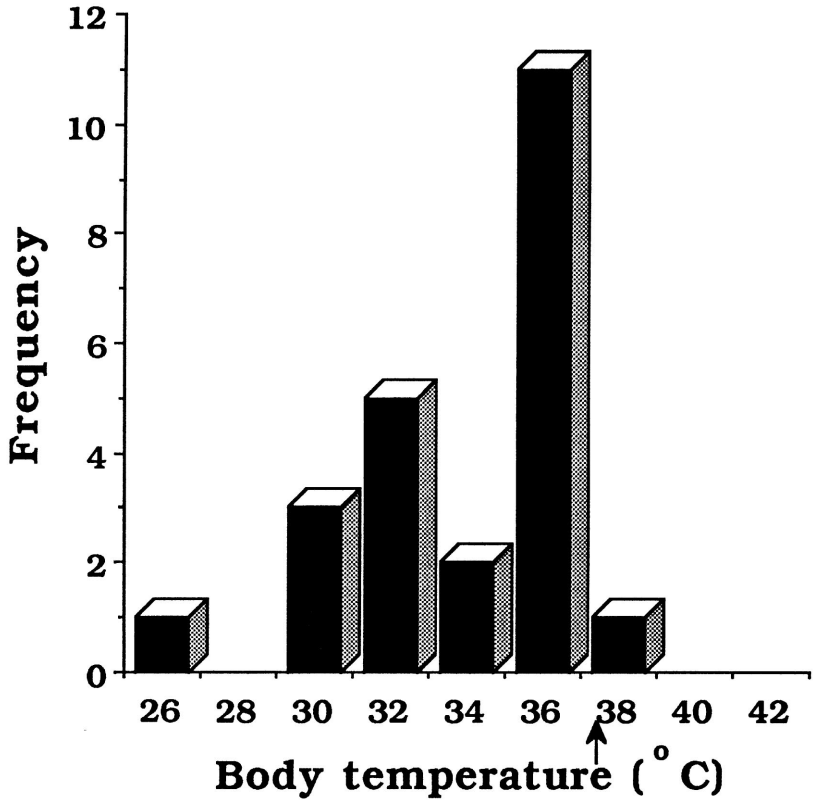

Fig. 1. Distribution of body temperatures of *Proarna bergi* recorded when endogenous warm-up behavior ceased. The bimodal distribution of warm-up temperatures may represent the body temperature range of reproductive activity. The thermoregulatory maximum voluntary tolerance temperature measured in the laboratory is indicated by the arrow.

Proarna bergi and *P. insignis* were found to use endogenous heat production to remain active under adverse environmental conditions. We observed populations of both species singing under heavy overcast, during rainy conditions, and at dusk. The lack of solar radiation coupled with environmental conditions means that the elevated T_bs observed in these species were a result of endogenous heat production. Recorded T_bs in *P. insignis* were as much as 7.4°C greater than T_a when solar heating was unavailable to the species.

Discussion

Proarna insignis was originally described from Nicaragua and Panama (Distant 1881). Its range extends from Mexico in the north (Jacobi 1907) to northwestern Argentina where it inhabits grasses in clearings in the cloud forest.

Torres (1961) synonymized *P. bergi* (Distant) with the closely related *P. bufo* Distant. Our collections in Argentina show two distinct varieties or subspecies. The

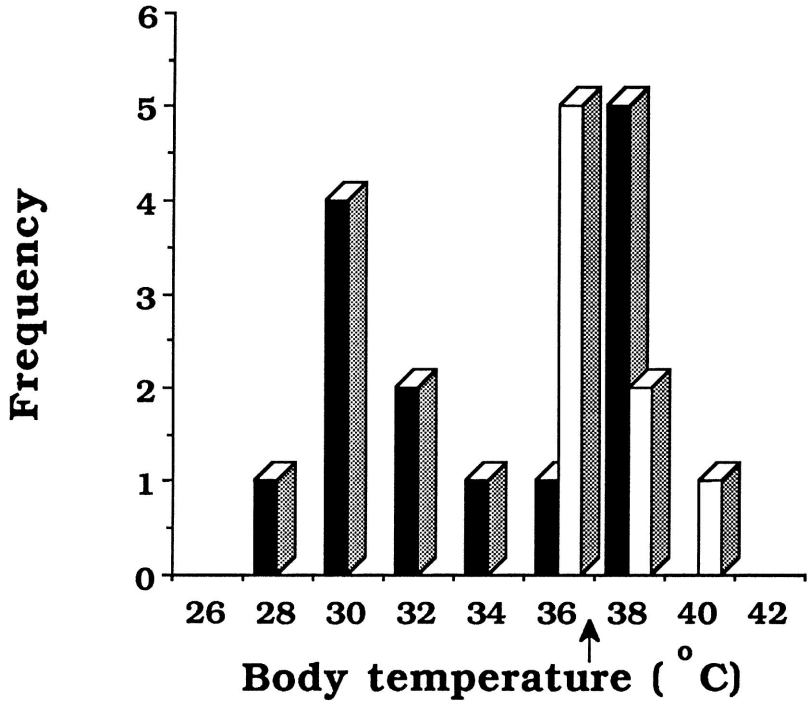

Fig. 2. Body temperature distribution of singing *Proarna insignis* (open columns) and the body temperature distribution when voluntary endogenous warm-up ceased in the laboratory (closed columns). A bimodal distribution of warm-up temperatures is clearly evident and probably corresponds to the temperature range of reproductive activity. There is a clear correspondence between the upper peak of the warm-up temperatures with body temperatures recorded in the field. The upper thermoregulatory temperature measured in the laboratory is indicated by the arrow.

southern variety corresponds to the type of *P. bufo* in the British Museum. We did not observe endothermic behavior in this group. The northern variety is found in open grasslands from the province of Cordoba and the northern edge of Buenos Aires province in the south northward throughout Argentina. Its range also extends marginally into the cloud forests of northwestern Argentina. The limits of its distribution in South America have not been established. We considered this northern variety to be the original *P. bergi* and have restricted the data presented here to that variety.

In most cicadas T_b increases are accomplished by behaviorally altering the heat gain from solar radiation (Heath 1967; Heath & Wilkin 1970; Sanborn et al. 1992). *P. bergi* and *P. insignis* are similar to ectothermic cicadas in that they use radiant solar energy to raise T_b for activity. The *Proarna* species will bask at low T_bs and augment this solar heating with metabolic heat production as has been described in other endothermic cicadas (Sanborn et al. in press). The endogenous heat production of the *Proarna* species is generated and used for thermoregulation. The endothermy described here is not simply the result of activity in an isolated, active body region, e.g. the timbal muscles, as has been described by Josephson & Young (1979; 1985).

At dusk or when environmental conditions prevent the utilization of solar radiation, *P. bergi* and *P. insignis* use metabolic heat to become active. These cicadas produce metabolic heat only when necessary and can be termed facultative or partial endotherms. Restriction in the use of metabolic heat saves energy stores in the cicadas. The energetic expense of behavioral thermoregulation is the cost of transporting the animal mass from one location to another or the cost of maintaining a particular posture. The energetic expense of behavioral thermoregulation represents a small fraction of the energy cost to maintain T_b using metabolic heat (Heath 1970). Conservation of energy is very important in cicadas since they have limited access to nutrients in the xylem fluid on which they feed (Cheung & Marshall 1973).

The cooling rate is greater than, and the estimated V_{O_2} less than (Table 2), the values determined for other endothermic cicada species (Bartholomew & Barnhart 1984; Sanborn et al. in press). The differences are expected due to the smaller size of the *Proarna* species. The values we obtained by indirect methods scale as expected when compared to the values reported for larger cicadas (Bartholomew & Barnhart 1984; Sanborn et al. in press).

The thermal responses of cicadas can be related to their activity patterns and their habitat. Table 3 summarizes the reported cicada temperature responses. The endothermic cicadas generally have a lower MFT and a greater temperature range of full activity than the ectothermic species. Heath et al. (1972) suggested the MFT probably relates more to the physical design of the cicada flight motor system than to the origin of the cicada. However, the low MFTs of the endothermic cicadas may be necessary for dusk activity. A low MFT permits the cicada to use the heat produced in flight to raise T_b. If the MFT were elevated, activity could possibly be inhibited by low T_a. The lower MFT of *P. insignis* may also be related to the cloud forest habitat of the species.

The temperatures of thermoregulatory responses determined for the *Proarna* species are higher than the those determined for the other endothermic cicadas. Both species of *Proarna* are diurnally active so the potential of radiant solar heat gain may necessitate a higher MVT than found in other endothermic cicadas. The other endothermic cicadas either have a brief period of activity in the morning hours, specialize in dusk activity, or are found in deep tropical forests and are protected from temperature extremes (Sanborn et al. in press).

The temperature of heat torpor in *P. bergi* is greater than in *P. insignis*. *P. bergi* may require a higher heat torpor temperature in the grassland in which the species lives. The open grassland is susceptible to extremes of temperature that may not occur in the cloud forest habitat of *P. insignis*. Since *P. insignis* is probably not exposed to temperature extremes in the cloud forest, *P. insignis* can survive with a lower heat torpor temperature.

Metabolic heat for thermoregulation is generated by the flight musculature in cicadas. Bartholomew & Barnhart (1984) described non-flapping warm-up occurred with “barely visible wing movements of low frequency” in *Fidicina mannifera*. Sanborn et al. (in press) describe two cicada species using heat generated during flight for endothermic temperature regulation. Metabolic heat in *P. bergi* and *P. insignis* was produced by rapid shiver-like movements of the wings and is a new mechanism of heat production not previously described in cicadas. The amplitude of the wing vibrations did not change noticeably with increasing T_b as is found in some moths (Dorsett 1962; Kammer 1981). The mechanism producing the wing vibrations was probably similar to the near synchronous activation of wing elevator and depressor muscles described in Lepidoptera (Kammer 1968; 1970; Kammer & Rheuben 1976) and bees (Esch & Goller 1991).

The relationship between field temperatures and the T_b when voluntary endogenous heat production ceased in *P. bergi* and *P. insignis* presents good evidence that the

TABLE 3. MEAN TEMPERATURE RESPONSES (°C) REPORTED IN THE LITERATURE FOR CICADAS. ENDOTHERMIC SPECIES ARE MARKED WITH AN ASTERISK (*).

Species	Minimum Flight Temperature	Maximum Voluntary Tolerance	Heat Torpor	Range of Full Activity
Proarna bergi[1]*	20.7	37.6	46.3	25.6
Proarna insignis[1]*	19.3	36.4	44.0	24.7
Dorisiana bonaerensis[2]*	16.7	34.6	44.6	27.9
Quesada gigas[2]*	19.1	33.8	44.9	25.8
Fidicina mannifera[2]*	19.8	32.0	42.0	22.2
Magicicada cassini[3,4]	20.9	31.8	43.0	22.1
Diceroprocta apache[5]	21.9	39.2	45.6	23.7
Cacama valvata[6]				
Camp Verde	23.7	37.3	44.6	20.9
Agua Fria	24.0	34.9	44.3	20.3
Okanagana hesperia[7]	22.9	36.3	43.5	20.6
Okanagodes gracilis[8]	22.7	41.2	48.7	26.0

[1]Present study.
[2]Sanborn et al., in press.
[3]Heath, 1967.
[4]Heath et al., 1971.
[5]Heath and Wilkin, 1970.
[6]Heath et al., 1972.
[7]Heath, 1972.
[8]Sanborn et al., 1992.

cicadas are warming to a level necessary for activity. The animals generally cease warm-up behavior in the same T_b range of animals active in the field (Fig. 2). The data also illustrate that the animals possess the metabolic machinery necessary to raise their T_b to a biologically significant range. The T_b that the *Proarna* species cease warm-up behavior in the laboratory is equal to the modal singing temperatures recorded in the field. This suggests the regulation of T_b through endogenous heat production.

Reproductive adult cicadas live for a limited time period, usually 6-8 weeks. Endothermy in the *Proarna* species may serve to increase reproductive fitness by uncoupling reproductive behavior from possible physiological constraints imposed by the environment. The ability to produce metabolic heat circumvents a reliance on environmental conditions to determine when an animal may or may not be active. Decreases in solar radiation by clouds or rain causes non-endothermic cicadas to decrease or suspend activity (Alexander & Moore 1958; Sanborn 1990; Sanborn & Phillips 1992). *P. bergi* and *P. insignis* can initiate activity and remain active during heavy overcast and/or rainstorms by using endogenous heat production thereby increasing the duration of activity on a given day and the number of days they are potentially active. Endothermy may permit *P. insignis* to inhabit the cloud forest. The seemingly ever present clouds and fog could inhibit reproductive activity in an ectothermic species for the majority of the adult life cycle. If environmental conditions were to prevent reproductive activity, the local population would become extinct.

Endothermy may also decrease predation on *P. bergi* and *P. insignis* populations. Acoustic insects represent a potential food source that advertise their location to po-

tential predators with the calling song. Many predators have been shown to use the reproductive signals of insects in locating their prey (summarized in Bailey 1991). The endothermic species can decrease predation pressure on the population by calling when environmental conditions decrease the hunting success of predators, e.g. at dusk.

A second manner in which endothermy may decrease predation in *P. bergi* and *P. insignis* populations deals with their choice of habitat. The thick grass and intertwining vegetation of their habitats at times made capturing a specimen difficult. In certain locations the use of an insect net was impossible. The easiest way to locate a singing cicada in these habitats was to compress the grass where the animal was singing. By orienting on the alarm call emanating from the compressed grass, the specimen could be localized and captured. By singing from deep within the grass, the *Proarna* species decrease their exposure to potential predators and decrease the risk of predation.

The *Proarna* species use endogenous heat production to become and/or to remain reproductively active. They regulate endogenous heat production through an on-off control system to elevate and maintain a T_b within the range required to coordinate the physiological processes of reproduction.

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ARTHROPODS ASSOCIATED WITH *ANNONA* SPP. IN THE NEOTROPICS

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ABSTRACT

Two hundred and ninety-six species of arthropods are associated with *Annona* spp. The genus *Bephratelloides* (Hymenoptera: Eurytomidae) and the species *Cerconota anonella* (Sepp) (Lepidoptera: Oecophoridae) are the most serious pests of *Annona* spp. Host plant and distribution are given for each pest species.

Key Words: *Annona*, arthropods, Insecta.

RESUMEN

Doscientas noventa y seis especies de arthrópodos están asociadas con *Annona* spp. en el Neotrópico. De las especies mencionadas, el género *Bephratelloides* (Hymenoptera: Eurytomidae) y la especie *Cerconota anonella* (Sepp) (Lepidoptera: Oecophoridae) sobresalen como las plagas mas importantes de *Annona* spp. Se mencionan las plantas hospederas y la distribución de cada especie.

The genus *Annona* is confined almost entirely to tropical and subtropical America and the Caribbean region (Safford 1914). Edible species include *Annona muricata* L. (soursop), *A. squamosa* L. (sugar apple), *A. cherimola* Mill. (cherimoya), and *A. reticulata* L. (custard apple). Each geographical region has its own distinctive pest fauna, composed of indigenous and introduced species (Bennett & Alam 1985, Brathwaite et al. 1986, Brunner et al. 1975, D'Araujo et al. 1968, Medina-Gaud et al. 1989, Peña et al. 1984, Posada 1989, Venturi 1966). These reports place emphasis on the broader aspects of pest species. Some recent regional reviews of the status of important pests and their control have been published in Puerto Rico, U.S.A., Colombia, Venezuela, the Caribbean Region and Chile (Medina-Gaud et al. 1989, Bustillo & Peña 1992, Peña et al. 1984, Gutierrez & Trochez 1977, Venturi 1966, Anonymous 1989). There is no contemporary published summary of Neotropical arthropods of *Annona* spp.. This list is offered as a contribution to an understanding of the arthropod fauna of *Annona* spp. in the Neotropics.

In the following checklist, distributional information is given for each arthropod. The checklist was compiled from collections of arthropods by the authors in Colombia, Ecuador, Brazil, and Dominican Republic, by summarizing the reports from several publications (Venturi 1966, Gutierrez & Trochez 1977, Medina-Gaud et al. 1989, Marin 1973, D'Araujo et al. 1968, Bennett & Alam 1985, Alata 1973, Anguita 1990,

Brussel & Wiedjk 1975, Deckle 1965, Fennah 1937, Grissell & Schauff 1990, Hamon & Williams 1984, Kimball 1965, Mound & Halsey 1978, Peña et al. 1984, Russell 1948, Hill 1975, Ebeling 1959) and by obtaining information from the collection of arthropods in Florida, USA, Honduras and Mexico and collections in Ecuador and Colombia by the authors.

The first column of the checklist refers to the *Annona* species. Arthropod order, family, genus and species are listed and the arthropod distribution by country based on *Annona* spp. records is given. The versions of the scientific names used in this text may not be the same as those used in the references.

DISCUSSION

The arthropod families most frequently observed on *Annona* spp. were Coccidae (Homoptera), Noctuidae, Oecophoridae (Lepidoptera), Eurytomidae (Hymenoptera) and Nitidulidae (Coleoptera). The most common arthropod species in the different neotropical countries are *Bephratelloides cubensis*, *B. pomorum*, *Cerconota anonella*, *Parasaissetia nigra*, *Saissetia coffeae*, *S. oleae* and *Cocytius antaeus*. The first three species are considered key pests of *Annona* spp. They are stenophagous, multivoltine *Annona* seed and fruit feeders. *Bephratelloides cubensis* has been reported from the USA, Caribbean Region, Central America and northern South America, whereas *B. pomorum* is listed from Central and South America. Biology and habits of *Bephratelloides* spp. have been provided by Brunner & Acuña (1967), Peña et al. (1984) and Nadel & Peña (1991). *Cerconota anonella* has been reported from tropical America and the Caribbean Region. Information on its biology, morphology and control is provided by Bustillo & Peña (1992). The last two genera are secondary pests of *Annona* spp. *Saissetia* spp. are polyphagous, leaf and branch feeders. *C. anteus* is a stenophagous species collected from the USA, the Caribbean and northern South America. Species of the family Nitidulidae were collected from flowers and decaying fruits. Nitidulids are considered important pollinators of *Annona* spp. (Gazit et al. 1982, Podoler et al. 1985, Nagel et al. 1985). This preliminary list of the arthropod fauna of *Annona* in the Neotropics points out that, although the distributions and relationships of the *Annona* are still poorly known, *Annona* spp. host a complex number of species throughout the Neotropical area. Since *Annona* spp. are mostly confined to the Neotropics, we would expect a lower number of stenophagous species in those areas where *Annona* was introduced (i.e., Asia, Australia).

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TABLE 1. LIST OF ARTHROPOD PESTS OF *ANNONA* SPP. IN THE AMERICAS AND THE CARIBBEAN REGION.

Annona[a] Species	Order	Family	Species	Country[b]
15	Isoptera	Kalotermitidae	*Neotermes* sp	8
9		Termitidae	*Nasutitermes costalis* (Holmgren)	21
15	Thysanoptera	Phlaeothripidae	*Liothrips anonae* Moulton	13
16		Thripidae	*Frankliniella bispinosa* (Morgan)	1
16			*F. kelliae* Sakimura	1
1,2			*Heliothrips haemorrhoidales* (Bouche)	13,11
15			*Selenothrips rubrocinctus* (Giard)	8
2			*Thrips tabaci* Lindeman	11
1	Orthoptera	Gryllidae	*Oecanthus niveus* (De Geer)	2
9	Hemiptera	Coreidae	*Leptoglossus zonatus* (Dallas)	17,2
9			*L. phyllopus* (L.)	2
2			*Madura perfida* Stal	17
9			*Sphictyrtus chrysis* (Lich)	17
9		Cydnidae	*Cyrtomenus* sp.	3
2		Lygaeidae	*Neopamera bilobata* (Say)	17
9		Miridae	*Hyaloides mani* Maldonado	21
9			*Monalonium* sp.	17
2			*Phytocoris* sp.	9

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TABLE 1. (CONT.) LIST OF ARTHROPOD PESTS OF *ANNONA* SPP. IN THE AMERICAS AND THE CARIBBEAN REGION.

Annona[a] Species	Order	Family	Species	Country[b]
1,15		Pentatomidae	*Antiteuchus tripterus* (F.)	8
9			*Edessa vinula* Stal	17
9			*Loxa flavicollis* Drury	7
15			*Mecistorhinus amplus* (Walker)	13
9,11		Pyrrhocoridae	*Dysdercus fernaldi* Ballou	17
2		Reduviidae	*Sinea coronata* Stal	17
1		Tingidae	*Corythucha gossypii* (F.)	17,18,21
9			*Corythucha* sp.	2
3			*Idiostyla anonae* (Drake & Hambleton)	13
3			*Teleonemia morio* (Stal)	13
1,2,9	Homoptera	Aetalionidae	*Aethalium reticulatum* (L.)	2,8,17
1,9,15		Aleyrodidae	*Aleurodicus cocois* (Curtis)	13
1			*A. dugesii* Cockerell	23
15			*A. dispersus* Russell	1
12,15			*A. neglectus* Quaintance & Baker	6,13,16
2,9,12,15			*Aleurocanthus woglomi* Ashby	18,21,22
2,12			*Aleurotrachelus trachoides* (Back)	10,18

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Annona[a] Species	Order	Family	Species	Country[b]
1			*Aleurotrachelus* spp.	8
1,12			*Aleurothrixus floccosus* (Maskell)	11,13
15			*Ceraleurodicus moreirai* Costa Lima	13
1,2			*Lecanoideus giganteus* (Quantance & Baker)	8,13,16
15			*L. mirabilis* (Cockerell)	8,23
13			*Metaleurodicus lacerdae* (Signoret)	13
9			*Tetraleurodes* sp.	23
5,15			*Trialeurodes floridensis* (Quaintance)	9,4
9		Aphididae	*Aphis citricola* Van de Groot	21
1,2,16			*A. gossypii* (Glover)	1,8,10
1,9			*A. spiraecola* Patch	17,8
1,15			*Macrosiphum euphorbiae* (Thomas)	8
8,9			*Toxoptera aurantii* (Foyer de Fonscolombe)	13,17,21
1			*Toxoptera citricida* (Kirkaldy)	8
9			*Toxoptera* sp.	2
1,3,15		Asterolencanidae	*Asterolecanium pustulans* (Cockerell)	13,18,21
9		Cercopidae	*Clastoptera* sp.	2
9		Cicadellidae	*Erthrogonia quadriguttata* (F.)	17

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TABLE 1. (CONT.) LIST OF ARTHROPOD PESTS OF *ANNONA* SPP. IN THE AMERICAS AND THE CARIBBEAN REGION.

Annona[a] Species	Order	Family	Species	Country[b]
9			*Graphocephala propior* (Fowler)	17
9			*G. spinosa* Van Duzee	17
9			*Oncometopia* sp.	17
15		Coccidae	*Ceroplastes actiniformis* Green	13
15			*C. cirripediformis* Comstock	1
1,15			*C. deodorensis* Hempel	13
8,15			*C. floridensis* Comstock	1,13
15			*Ceroplastes* sp.	13
8,9,12,14,15			*Coccus longulus* (Douglas)	1,8,9
8,12			*C. viridis* (Green)	1
15			*C. hesperidum* L.	1
2,9,12			*Eucalymnatus tessellatus* (Signoret)	1
1,2,8,9,11,15			*Parasaissetia nigra* (Nietner)	3,8,13,17,18,21
2			*Parthenolecanium corni* (Bouché)	10
9,15,16			*Philephedra tuberculosa* Nakahara & Gill	1,21
15			*Platingisia noacki* Cockerell	13
12,15			*Protopulvinaria pyriformis* (Cockerell)	1,8
9			*Pulvinaria psidii* Maskell	1

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TABLE 1. (CONT.) LIST OF ARTHROPOD PESTS OF *ANNONA* SPP. IN THE AMERICAS AND THE CARIBBEAN REGION.

Annona[a] Species	Order	Family	Species	Country[b]
15			*P. urbicola* Cockerell	1
1,15			*Saissetia anonae* Hempel	13
1,6,9,11,12,15			*S. coffeae* (Walker)	3,8,13,17,18,21
9			*S. oleae* (Olivier)	1,21,17
9			*Vinsonia stellifera* (Westwood)	17
2		Conchaspididae	*Conchaspis agreci* Cockerell	10
2,5		Diaspididae	*Abgrallaspis cyanophylli* (Signoret)	1
2			*Acutaspis scutiformis* (Cockerell)	10
1,9			*Aonidiella aurantii* (Maskell)	13,21
9			*A. comperei* McKenzie	13,21
1,5,6,12			*A. orientalis* (Newstead)	1
1,2,9,12			*Aspidiotus destructor* Signoret	1,8,13
2,12			*A. nerii* Bouché	1,11
15			*A. similimus translucens* (Cockerell)	13
15			*Aspidiotus* sp.	13
1,9			*Chrysomphalum aonidum* L.	13,21
1,9			*C. dictyospermi* (Morgan)	13,18
1			*Clavaspis herculeana* (Doane & Hadden)	13

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TABLE 1. (CONT.) LIST OF ARTHROPOD PESTS OF *ANNONA* SPP. IN THE AMERICAS AND THE CARIBBEAN REGION.

Annona[a] Species	Order	Family	Species	Country[b]
2,9,12			*Hemiberlesia lataniae* Signoret	1,10
1			*Howardia biclavis* (Comstock)	1
1			*Ischnaspis longirostris* (Signoret)	13,8
2			*Lepidosaphes beckii* (Newman)	10
9			*Lepidosaphes* sp.	2
9			*Lindisgaspis floridana* Ferris	1
1			*Melanaspis paulista* (Hempel)	13
15			*M. mimosae* (Comstock)	1
9			*Mycetaspis personata* (Comstock)	21
12			*Palinaspis quohogiformis* (Merill)	1
1,15			*Pinnaspis aspidistrae* (Signoret)	8,13
1,9,15			*P. strachani* (Cooley)	1,8,13,21,27
9			*Pinnaspis* sp.	2
1			*Pseudalacaspis acephala* Ferris	8
1,15			*P. sordida* Hempel	13
1			*Pseudadonidia trilobitiformis* (Green)	8,13
8,6			*Pseudischnaspis bowreyi* Cockerell	1,18
9			*Radionaspis indica* (Marlatt)	1

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Annona[a] Species	Order	Family	Species	Country[b]
1,2,9			*Selenaspidus articulatus* (Morgan)	1,17,18,21
2,9			*Unaspis citri* (Comstock)	3,10
9		Flatidae	*Poekiloptera* sp.	2
1		Margarodidae	*Crypticerya rosae* Riley & Howard	8
2			*Icerya purchasi* Maskell	10,11
1			*I. montserratensis* Riley & Howard	8
1		Membracidae	*Aconophora concolor* Walker	8
9			*A. pugionata* Germar	13
1,2,9			*Aconophora* sp.	2,3,8,17
1			*Boethoos reticulata* (F.)	8
1			*Bolbonata insignis* Fowler	8
2,9,11			*Campylenchia hastata* Stal	17
2			*Ceresa vitulus* (Fabricius)	17
2			*Cyphonia clavata* (Fabricius)	17
2			*Enchenopa lanceolata* (Stol)	17
1			*Encophyllum quinquemaculata* (Fairmoire)	13
1			*Erechtia* sp.	8
1			*Gerridius scutellatus* Fowler	8

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Annona[a] Species	Order	Family	Species	Country[b]
1			*Guayaquila* sp. near *noreriana* Goding	8
1			*Holophorium* sp.	8
1,2,9,11,15			*Horiola picta* (Cockerell)	2,17
15			*Membracis albolimbata* Fowler	17
1,9			*M. arcuata* De Geer	13
9			*M. foliata* (Richter)	8,13,17
2,9,11			*M. mexicana* Guerin	3,8
1			*Metcalfiella monogramma* Germar	8
9			*Scictopelta acutula* (Fairmont)	17
1,2,9			*Tragopa involuta* (F.)	8
9			*Vanduzea laeta segmentata* (Fowler)	17
1,9		Ortheziidae	*Orthezia insignis* Browne	2,13,17
2			*O. olivicola* Beingolea	10
15		Pseudococcidae	*Dysmicoccus brevipes* (Cockerell)	13
1,15			*Ferrisia virgata* (Cockerell)	8,13
9,15			*Nipaecoccus nipae* (Maskell)	13,18,21
1			*Nipaecoccus* spp.	8
1,9,15,16			*Planococcus citri* (Risso)	1,7,8,13

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Annona[a] Species	Order	Family	Species	Country[b]
2			*Pseudococcus calceolaria* (Maskell)	11
1,2,9			*P. longispinus* (Targioni)	11,13,21
2			*P. maritimus* (Ehrhorn)	2,9
1,9,16			*Pseudococcus* spp.	1,8,12,13
1			*Pseudotectococcus anonae* Hempel	13
2	Lepidoptera	Gracillariidae	*Phyllocnistis* sp.	8,9,10,17
1		Hesperiidae	*Bungalotis fulvius* (Plotz)	13
9			*Camptopleura* spp.	17
1			*Discophellus ramusis* (Stoll)	13
1,9			*D. sebaldus* (Stoll)	13
1,9			*Milanion hemes* (Cramer)	13
1			*M. leucaspis* Mabille	13
1,15			*Paraminus scurra* (Hubner)	13
1			*Pythonides herennius* (Geyer)	13
1			*Thracides scipio* (F.)	13
1,9,15		Lycaenidae	*Thecla ducalis* Doubleday	8,13,17
9			*T. irtyginus* Cramer	13
9			*T. ortygunus* Cramer	13

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TABLE 1. (CONT.) LIST OF ARTHROPOD PESTS OF *ANNONA* SPP. IN THE AMERICAS AND THE CARIBBEAN REGION.

Annona[a] Species	Order	Family	Species	Country[b]
1,2			*Thecla* sp.	10,13,18
2		Lyonetiidae	*Lyonetia* sp.	10
1			*Leucoptera* sp.	18
2,6,9,15,16		Noctuidae	*Gonodonta clotilda* (Stoll)	1,17,18
6,9,15,16			*G. nutrix* (Cramer)	1,18,24
15			*G. pyrgo* (Cramer)	13
1		Nymphalidae	*Mechanitis lysimnia* (F.)	13
1,9,12,15,8		Oecophoridae	*Cerconota anonella* (Sepp)	3,8,9,13,14,15,17,23
1,15			*Stenoma leucana* (Sepp)	8
15			*S. sciogama* Meyrick	13
2,9		Papilionidae	*Graphium ariarathes metagenes* (Rotschild & Jordan)	13
1,15			*G. harrisianus* (Swainson)	13
2,6			*G. lysithous* (Hubner)	6
1,2,9,15		Psychidae	*Oeiketicus kirbyi* (Guilding)	8,10,13,18
1,9			*Oeiketicus* sp.	2,8,17
15		Pyralidae	*Ectomyelois decolor* (Zeller)	13
1		Saturniidae	*Rhescyntis armida* (Cramer)	13
14			*Dirphia avia triangulum* Walker	13

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Annona[a] Species	Order	Family	Species	Country[b]
1			*Rothschildia aurota aurota* (Cramer)	13
2,11,14			*Hylesia coex* Dyar	17
1,9,15,16		Sphingidae	*Cocytius antaeus* (Drury)	13,17,21,24
1,15			*C. anteus medor* Stoll	13
15			*C. cluentius* (Cramer)	13
1,15			*C. duponchel* (Poey)	13,18
9			*Protambulix strigilis* Clark	22
1	Coleoptera	Anobiidae	*Catorama palmarum* (Guerin-Meneville)	8
9		Anthribidae	*Araecerus fasciculatus* De Geer	8
1		Cerambycidae	*Alphus canescens* (Bates)	13
15			*A. senilis* Bates	13
1			*Ammiscus polygraphoides* White	10
1			*Ancylocera* sp.	13
1			*Ibidion* sp.	8
15			*Oncideres aegrota* Thomson	13
15			*O. dejeani* Thomson	13
15			*O. saga* (Dalman)	13
1			*Trachyderes interruptus* Dupont	8

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Annona[a] Species	Order	Family	Species	Country[b]
9			*Trachysomus thomsoni* Aurivillius	17
2		Chrysomelidae	*Alethaxius* sp.	17
2			*Colaspis lebasi* Lefebre	17
2			*Diabrotica centralis* Jacoby	17
2			*D. mutabilis* Baly	17
2			*Diphaulaca panamae* Barber	17
1			*Justus gibber* Bondar	13
1			*J. tuberosus* Bondar	13
2,9			*Megascelis* sp.	17
1			*Uroplata anonicola* Maulik	13
6,11		Curculionidae	*Brachiomus quadrinodosus* (Boheman)	17
2,8			*Compsus signatus* Say	17
3			*Conotrachelus anonae* Bondar	13
15			*C. bondari* Marshall	13
3			*C. marshalli* Bondar	13
1,9,10,15			*Cratosomus bombina* F.	8,13,17
1,15			*Cratosomus* sp.	13
1			*Heilus ochrifer* (Boheman)	13

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Annona[a] Species	Order	Family	Species	Country[b]
15			*Helipus catagraphus* Germar	13
15			*H. lactarius* Germar	13
1,15			*H. velamen* (Boheman)	13
1			*Lydamis angulus* Boheman	13
1			*L. confusa* Casey	13
1			*L. maculata* Casey	13
1			*L. ruficollis* Bondar	13
1			*Lydamis* sp.	13
2			*Naupectus xanthographus* (Germar)	11
3,10,15			*Priomerus brevirostris* Hustache	13
1,9			*Prionumerus flavitarsis* Jekel	17
1			*Spermologus anonae* (Araujo)	13
3			*S. funerus* (Pascoe)	13
1			*Telemus carinosus* (Casey)	13
1,3			*T. gibbicollis* (Casey)	13
2		Elateridae	*Conoderus* spp.	11
9,16		Nitidulidae	*Carpophilus fumatus* (Boheman)	1,13
16			*C. freemani* (Dobson)	1

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Annona[a] Species	Order	Family	Species	Country[b]
9,16			*C. mutilatus* (Erichson)	1,8,21
9,16			*C. humeralis* (F.)	1,8
9			*C. succisus* (Erichson)	8
2			*Colopterus macropterus* (F.)	9
16			*C. posticus* (Erichson)	1
2			*C. vulneratus* (Erichson)	9
9			*Colopterus* sp.	8
16			*Haptoncus luteolus* (Erichson)	1
16			*Lobiopa* sp.	1
6		Languridae	*Hapalips* prob. *fusca* Reiter	8
15		Scolytidae	*Coccotrypes pygmaeus* Eichhoff	13
16			*Pityophilus* sp.	1
15,16			*Xyleborus* sp.	1,13
1,15		Scarabaeidae	*Ancistrosoma klugi* Curtis	10
15			*Antichira inaurata* Burmeister	8
9			*Cyclocephala brittoni* Endrodi	8
1,15			*C. ruficollis* Burmeister	8
15			*Macrodactylus affinis* Laporte	3

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Annona[a] Species	Order	Family	Species	Country[b]
15			*M. pumilio* Burmeister	13
15			*M. suturalis* Manneheim	13
9		Staphylinidae	*Coproporus* sp.	8
9	Diptera	Anthomyiidae	*Atherigona orientalis* (Schiner)	17
9		Bibionidae	*Plecia plagiata* Wiedeman	17
1		Lonchaidae	*Lonchea aculeata* Bezzi	8
1			*Neosilba* sp.	8
1			*Silba dimidiata* (Curran)	8
1,9,15			*S. pendula* (Bezzi)	8,13,17
2		Tephritidae	*Anastrepha bistrigata* Bezzi	10
2			*A. barandiaranae* Korytowski & Ojeda	9
2			*A. chiclayae* Greene	10
2			*A. distincta* Greene	10
2			*A. extensa* Stone	10
2			*A. fraterculus* (Wiedemann)	9,10,13
2			*A. obliqua* (Macquart)	13
2			*A. scholae* Capoor	13
2			*A. serpentina* (Wiedemann)	10

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2,9			*A. striata* Schiner	2,10,17
2			*A. suspensa* (Loew)	10
1,2			*Anastrepha* sp.	8,13
2,9			*Ceratitis capitata* (Wiedemann)	2,13
2			*Tomoplagia monostigma* Hendel	10
2			*Trupanea fucata* F.	10
2			*Xanthaciura major* Malloch	1
1,2,6,8,9,12,15,16	Hymenoptera	Eurytomidae	*Bephratelloides cubensis* (Ashmead)	2,3,4,6,7,8,17,18,20, 21,22,25,26
1			*B. limai* (Bondar)	13
1			*B. paraguayensis* Crawford	12,24
1			*B. petiolatus* Grissell & Schauff	7
1,2,8,9,15			*B. pomorum* (F.)	2,3,5,6,7,8,9,10,17,1 8,20,21,22,26,26
15		Formicidae	*Atta sexdens rubropilosa* Forel	13
15			*A. sexdens sexdens* (L.)	13
9			*Atta* sp.	2,17
14			*Camponotus* sp.	2

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14			*Dolichoderus bispinosus* (Olivier)	13
1			*Monacis trinidadensis* Provancher	8
1			*Monomorium floricola* (Jerdon)	1
1			*Paratrechina longicornis* (Latreille)	1
14		Meliponidae	*Trigona trinidadensis silvestriana* Vach.	13
14			*Trigona* sp.	2,13
14		Vespidae	*Polistes versicolor* Olivier	13
15	Acarina	Eriophyidae	*Eriophyes anonae* Keifer	8
2		Tenuipalpidae	*Brevipalpus chilensis* Baker	11
2			*B. californicus* (Banks)	11
2		Tetranychidae	*Oligonychus yothersi* (McGregor)	11
14			*Oligonychus* sp.	3
2			*Tetranychus urticae* Koch	11
15			*Tetranychus mexicanus* (McGregor)	8

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GROWTH OF THE MITE *MONONYCHELLUS TANAJOA* (ACARI: TETRANYCHIDAE) ON ALTERNATIVE PLANT HOSTS IN NORTHEASTERN BRAZIL

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Cassava, *Manihot esculenta* Crantz (Euphorbiaceae), is one of the main food crops cultivated by small land holders in tropical areas of South America and Africa (CIAT 1990). It is also grown on a large scale in Thailand, Indonesia and several other Asian countries. The cassava green mite, *Mononychellus tanajoa* (Bondar), is one of the most destructive pests of this crop in parts of South America and Africa where it can cause yield losses of up to 80%, depending mostly upon the age of the plants when the heaviest attacks occur (CIAT 1976, Veiga 1985, Yaninek & Herren 1988).

M. tanajoa has been commonly considered as an oligophagous species restricted to attacking plants of the genus *Manihot*, and its biology has been studied only on cassava leaves as food substrate (Murphy et al. 1984, Yaninek et al. 1989). However, Tuttle et al. (1977) reported the occurrence of *M. tanajoa* on *Cucurbita pepo* L. (Cucurbitaceae), *Lycopersicum esculentum* Mill (Solanaceae) and *Sechium edule* (Jacq.) (Cucurbitaceae) in the State of Ceará, northeastern Brazil, without referring to its actual feeding on those plants. Our unpublished field observations in several states of northeastern Brazil also reported it on *Passiflora cincinnata* Mart (Passifloraceae), *Manihot pseudoglaziovii* Pax. et K. Hoffm. (Euphorbiaceae), *Pavonia cancellata* Cav. (Malvaceae), *Solanum erianthum* D. Don. (Solanaceae), *Bauhinia forficata* Link (Caesalpiniaceae), *Borreria verticillata* G.F.W. Meyer (Rubiaceae), *Macroptilium martii* Benth. (Fabaceae), *Solanum paniculatum* L. (Solanaceae), *Emilia sonchifolia* DC. (Asteraceae) and *Canavalia brasiliensis* Mart. ex Benth. (Fabaceae). Apparently, *M. tanajoa* was only temporarily found on most of those plants after reaching very high numbers on cassava and dispersing by the wind to nearby vegetation.

However, all stages of *M. tanajoa* were repeatedly found on *P. cincinnata* and *M. pseudoglaziovii* even when the mite was not abundant on cassava, indicating that it was developing on those plants. Both *P. cincinnata* and *M. pseudoglaziovii* are common perennial, evergreen native plants of northeastern Brazil, found in large numbers in the cassava growing region.

The objective of this work was to compare the relative suitabilities of *P. cincinnata*, *M. pseudoglaziovii*, cassava (*M. esculenta*) and the leguminous common bean (*Phaseolus vulgaris* L.) as host plants of *M. tanajoa*. The last plant was chosen as a control on which *M. tanajoa* has never been reported, and on which it was not expected to perform well. The work was conducted under laboratory conditions in Petrolina, State of Pernambuco, at 24 ± 2 °C, 65 ± 10 %RH and a photoperiod of 13:11 (L:D).

Initially, 150 female *M. tanajoa* collected from cassava plants in Petrolina were transferred to rearing units made of pieces of cassava leaves (3 × 3 cm) placed lower side up on a foam mat maintained inside a plastic tray (20 cm in diam and 3 cm high) containing distilled water. The borders of the pieces of cassava leaves were covered with a band of cotton wool to prevent mites from escaping and to avoid leaf dehydration.

Eggs laid within 16 h were collected and isolated onto 80 rearing units made of mature, healthy leaves of each plant substrate tested. Determination of the duration of the immature stages was based on daily observations at 8:00 AM and 3:00 PM. Recently emerged adult females were paired with field-collected adult males. New males were introduced onto the rearing units whenever the previous ones died. Observations on oviposition were conducted daily at 8:00 AM, and progeny was reared to adulthood for sex ratio determination. Immatures and adults were transferred to new arenas every third day. Values of intrinsic rate of increase were determined by first calculating the approximate indexes, and then using Lotka's equation to correct the values (Birch 1948). One-way ANOVAs were used to detect significant treatment differences and Tukey's tests ($p < 0.05$) were used for comparisons of mean durations of the immature phase and of longevities.

Development from egg to adult occurred on all substrates tested. However, significant differences existed between plant treatments for both females ($F = 35.73$; df = 3,64, $p < 0.05$) and males ($F = 5.11$, df = 3,20, $p < 0.05$) in relation to duration of the immature phase (egg to adult). For females, duration on cassava (14.5 ± 0.2 days, mean ± standard) was not significantly different from duration on *M. pseudoglaziovii* (15.3 ± 0.4), these in turn were significantly shorter than on the other two plants. The duration in days on *P. cincinnata* (19.4 ± 0.5) was significantly longer than on common bean (17.3 ± 0.4). For males, the only significant difference was between the duration on *M. pseudoglaziovii* (12.7 ± 1.2) and *P. cincinnata* (16.8 ± 0.4).

Survivorship was considerably higher on cassava (79%) than on other plants (29 - 39%). Most mortality occurred at the protonymphal stage on both *P. cincinnata* and common bean. About 57% of the mites reaching adulthood on cassava were females, whereas on other plants, 74 - 78% were females (Table 1). As all mites came from the same source, this result indicates that the survivorship of males was more severely affected by the quality of the food source than the survivorship of females.

The longevity in days of adult females differed significantly among host plants (F = 25.40; df = 3,64, p < 0.05). Longevity on *P. cincinnata* (14.9 ± 1.5) was not significantly different from longevity on cassava (14.8 ± 1.0). Longevity on *M. pseudoglaziovii* (9.8 ± 1.1) was significantly shorter than longevity on the former plants, but longer than longevity on common bean (4.0 ± 0.9).

Mean generation time was longer on *P. cincinnata* (Table 1). Intrinsic rate of increase was considerably higher on cassava ($r_m = 0.14$), but *M. tanajoa* also developed and reproduced on *M. pseudoglaziovii* ($r_m = 0.09$) and *P. cincinnata* ($r_m = 0.06$). Total

TABLE 1. BIOLOGICAL PARAMETERS OF *MONONYCHELLUS TANAJOA* MITES ON DIFFERENT PLANT SPECIES.

Parameters	*Manihot esculenta*	*Manihot pseudoglaziovii*	*Passiflora cincinnata*
Mean generation time[1] (T)	24.7	21.2	30.2
Net reproductive rate (R)	25.1	6.2	5.3
Total fecundity (±SE)	41.8 ± 4.4	29.6 ± 3.7	19.5 ± 3.3
Sex ratio[2]	0.57	0.78	0.77
Intrinsic rate of increase[3] (r_m)	0.14	0.09	0.06

[1]Days
[2]Females/(Females + Males)
[3]Female/Female/Day

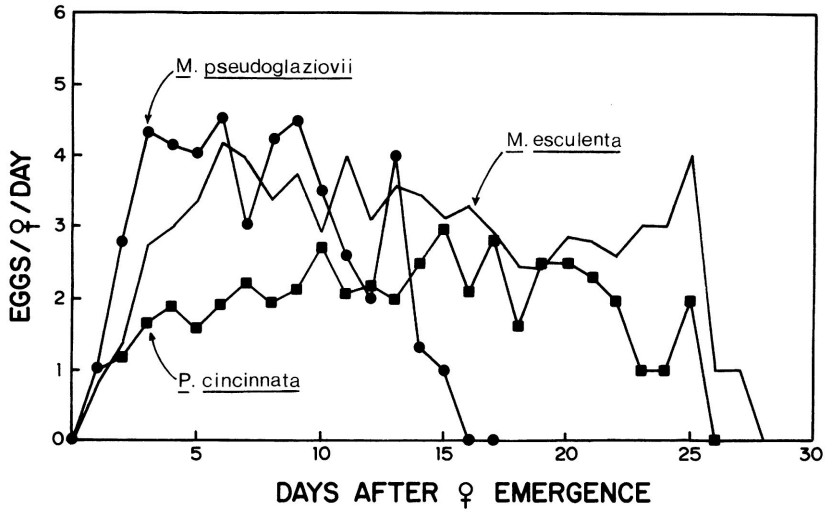

Fig. 1. Daily oviposition rates of the mite *Mononychellus tanajoa* on different plant substrates; calculations based on the total number of adult females present at the beginning of the observation period.

fecundity was lower on *P. cincinnata*, intermediate on *M. pseudoglaziovii* and highest on cassava. No oviposition occurred on common bean. Daily oviposition rates were about the same on cassava and *M. pseudoglaziovii* in the first 13 days of oviposition (Fig. 1), but they dropped rapidly to zero after that period on the latter and continued at high levels for another 12 days on the former. Oviposition rate on *P. cincinnata* was lower than on cassava, but the oviposition period was nearly as long. Eggs laid on *P. cincinnata* were considerably more fragile than on the two *Manihot* species, being disrupted very easily when handled with a brush.

Total duration of the immature phase of both sexes was comparable to results of previous studies (Murphy et al. 1984, Yaninek et al. 1989). Yaninek et al. (1989) studied the effect of season, cassava plant age and leaf age on the biology of *M. tanajoa*. They demonstrated that those factors did not significantly affect pre-adult development. The present study indicates that pre-adult development of this oligophagous mite can occur even on common bean, a plant which cannot sustain a population of that mite species. The practical significance of this result remains to be demonstrated because eggs are not oviposited on this host. Less preferred hosts could be important in maintaining *M. tanajoa* populations in the field. New colonies of tetranychid mites are known to start primarily with the arrival of dispersing pre-reproductive females onto new substrates and subsequent egg laying (Kennedy & Smitley 1985).

Yaninek et al. (1989) showed a significant effect of season, plant and leaf age on adults of *M. tanajoa*. As expected, the effect of the substrate on the adults was even more marked in the present study which compared different plant species. It was not surprising that *M. tanajoa* could reproduce on *Manihot* species other than cassava, as suggested by previous field surveys in northeastern Brazil (Bastos & Flechtmann 1985, Moraes & Flechtmann 1981). It was however unknown that it could reproduce on a plant species belonging to a different genus, *P. cincinnata*, commonly found in northeastern Brazil. Both of those plants are found in the cassava growing region throughout the year. The results of this study suggest the importance of those plants

as reservoirs of *M. tanajoa* and their possible role in maintaining local populations of that pest when cassava is not available or when its leaves are not physiologically suitable for the development of the mites. Conceivably, alternative host plants could be important in maintaining natural enemies of *M. tanajoa* in those periods, making it possible for them to reach the crop earlier and to increase their efficiency. Moraes et al. (1993) collected the 2 main predators of *M. tanajoa*, *Amblyseius idaeus* (Denmark & Muma) and *Amblyseius manihoti* Morae, on leaves of *P. cincinnata* in northeastern Brazil. Further studies should concentrate on evaluating the importance of that plant as an alternative substrate to those predators.

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Summary

The suitabilities of *Passiflora cincinnata*, *Manihot pseudoglaziovii*, *Manihot esculenta* (cassava) and *Phaseolus vulgaris* (common bean) were evaluated as food substrates for the mite *M. tanajoa*. The mite performed better on cassava (r_m = 0.14), although it also developed and reproduced on *M. pseudoglaziovii* (r_m = 0.09) and *P. cincinnata* (r_m = 0.06). Mites developed to the adult stage on common bean but did not oviposit.

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ANTIBIOSIS TO CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) IMMATURE STAGES IN CARAMBOLA GERMPLASM

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All cultivars of carambolas (*Averrhoa carambola* L.) grown in Florida are considered hosts of the Caribbean fruit fly [*Anastrepha suspensa* (Loew)] (Norrbom & Kim 1988). Fruits must be disinfested with cold, irradiation, or heat before they can be exported to nations having quarantine requirements. These treatments increase the cost of fruits and may decrease fruit quality. Resistant carambola cultivars have not been employed as a means for reducing losses to the pest by growers. Relaxation or elimination of postharvest quarantine treatment requirements may be possible for resistant cultivars.

Howard & Kenney (1987) determined that 'Golden Star' was less susceptible to Caribbean fruit fly than 'Arkin' or 'Fwang Tung' based on the numbers of eggs oviposited in harvested fruits. They ran their tests in cages using colony-reared flies and also found fewer and lower weights of pupae developing from 'Golden Star' fruits than from fruits of the other cultivars.

The present experiments were designed to identify antibiosis to Caribbean fruit fly eggs and larvae in some Florida carambola cultivar clones and seedlings from open pollinations or controlled crosses between various clones. Antibiosis (after Kogan & Ortman 1978) is due to fruit properties that adversely affect the metabolism of the growing fruit flies. Carambolas used in the experiments were from non-commercial research groves at the Miami National Clonal Germplasm Repository of the Subtropical Horticulture Research Station. Seedlings were derived from crosses described by Knight (1982) and Schnell & Knight (1989). Tree management included irrigation, fertilization, pruning, and mechanical and chemical weed control, but no insecticide application. Fruits were harvested between 16 December 1993 and 20 January 1994 as they became available.

Five clones and 11 seedlings described in Table 1 were bioassayed. A treatment consisted of one tree (cultivar or seedling) from one harvest date. For most clones and seedlings, only a single tree existed, therefore variability among trees was not assessed. 'Arkin' and 'Fwang Tung' were each bioassayed on two dates so effect of sam-

TABLE 1. CARAMBOLAS BIOASSAYED FOR ANTIBIOSIS TO CARIBBEAN FRUIT FLY AND MEAN (± SEM) PERCENTAGE ADULT EMERGENCE FROM FRUITS ARTIFICIALLY INFESTED WITH EGGS IN THE LABORATORY, MIAMI NATIONAL CLONAL GERMPLASM REPOSITORY, FLORIDA, 1993-94.

Treatment Cultivar/Seedling	Miami Accession No., Field Location	Emergence, %
'Golden Star' × 'Fwang Tung' seedling 7	25967, WA-4-4-11	61.0 ± 16.4a
'B-10'	26763, WB-1	43.0 ± 9.0ab
'Hew-1'	25129, WB-1	42.8 ± 13.3abc
'Fwang Tung', tested 20 Jan	22647, W2-2	42.0 ± 11.5abc
'Arkin', tested 20 Jan	25157, W2-2	42.0 ± 6.6abc
'Fwang Tung', tested 6 Jan	22647, W2-2	41.6 ± 17.2abc
'Golden Star' × 'Fwang Tung' seedling 5	25967, WA-4-3-32	40.0 ± 16.7a-d
'Golden Star' selfed seedling	25974, WA-4-2-32	32.0 ± 13.6a-e
'Arkin', tested 6 Jan	25157, W2-2	25.2 ± 7.2b-f
'Golden Star' × 'Fwang Tung' seedling 1	25967, WA-4-4-4	24.6 ± 5.0b-f
'Dah Pon' × open pollinated, seedling 1	25000, WA-4-2-6	24.0 ± 9.3b-f
'Golden Star' × 'Fwang Tung' seedling 3	25967, WA-4-4-18	21.6 ± 11.3b-f
'Golden Star' × 'Fwang Tung' seedling 2	25967, WA-4-1-134	16.4 ± 5.0b-f
'Demak'	21500, WA1-18-2	13.8 ± 7.5c-f
'Golden Star' × 'Fwang Tung' seedling 4	25967, WA-4-3-2	11.0 ± 6.0def
'Dah Pon' × 'Fwang Tung', seedling	25983, WA-4-2-134	5.4 ± 5.4ef
'Golden Star' × 'Fwang Tung' seedling 6	25967, WA-4-3-39	4.2 ± 4.2ef
'Dah Pon' × open pollinated, seedling 2	25000, WA-4-1-6	0f

Column means (n = 5) followed by the same letter do not differ ($P > 0.05$; Fisher's least significant difference test.)

pling date was also assessed for them. The other treatments were sampled on one date. Eighteen treatments, each comprising a cultivar or seedling and a harvest date, were bioassayed. Five fruits from one tree made up the five replicates of each treatment.

Bioassays were conducted on fruits within one h of harvest. Fruit quality was similar to that marketed from local packinghouses: at least 7.6 cm in length, 10-25% green in color (visual inspection), and free from rot, damage, and wild fruit fly infestations (visual inspection). A slice was cut from a fruit with a serrated knife; slices included peel and flesh, but not seeds. Slices served as the bioassay substrate and weighed 7.3 ± 1.3 g (mean ± SEM).

Caribbean fruit fly eggs used in bioassays were from a colony which had been maintained on an agar medium at the Miami USDA-ARS laboratory since 1971 (Hennessey 1994). Eggs were oviposited on waxed cheesecloth panels, washed from the panels with water, and incubated in a specimen chamber for three days (26 ± 2°C, 85 ± 10% RH, 13:11 [L:D], mean ± SEM) on paper towels moistened with an antifungal solution (sodium benzoate 0.07%).

Individual slices were placed in translucent, polypropylene specimen containers (120-ml). A piece of moist (antifungal solution) blotting paper containing 10 three-

day-old eggs was placed egg-side-down on the cut surface of each slice. The slice was misted with the antifungal solution (1 ml) and the container was sealed with a screw cap. Caps had a hole (1-cm diam) covered with organdy for ventilation. Controls consisted of agar diet (10 ml) inoculated with 10 eggs and misted with the antifungal solution. Five controls were bioassayed on each sampling date. Containers were placed in translucent, covered plastic boxes and held in the specimen chamber described above. Vermiculite (1 g) was placed in each container after four days to serve as a pupation medium. After seven days, pupae and prepupae were washed from the controls, placed in fresh vermiculite (1 g), and held until adult emergence in the specimen chamber. Prepupae and pupae were washed from treatments after 13 days (because they developed more slowly than controls) and held as above.

The percentage of eggs reaching adulthood from treatments divided by the percentage from the controls of the same sampling date was the criterion used for assessing antibiosis. The criterion was based on the report by Cowley et al. (1992) that defined a fruit fly host as a fruit from which reproductively viable adult flies could be reared from eggs oviposited into the fruit. Our method bypassed the oviposition process where antixenosis would have obscured antibiosis effects. PROC ANOVA (SAS Institute, 1992) was used to compare nontransformed percentages among the 18 treatments. Fisher's least-significant-difference (LSD) test ($P \leq 0.05$) was used to separate means after a significant ($P \leq 0.05$) F value was found. The SSN ranking selection procedure (Gibbons et al. 1974), which identifies the smallest subset that includes the best treatment, was also conducted as a separate test of means ($P \leq 0.05$).

Mean emergence varied between 0.0% and 61.0% among treatments (Table 1). Differences among treatments were significant ($F = 2.67$; df = 17, 72; $P = 0.002$). The 'Dah Pon' × open pollinated seedling 2, from which no flies emerged, was more antibiotic ($P \leq 0.05$; LSD) than 'Golden Star' × 'Fwang Tung' seedlings 5 and 7, 'B-10', 'Hew-1', 'Fwang Tung' (6 and 20 January), 'Arkin' (20 January), and the 'Golden Star' selfed seedling. It did not differ significantly ($P > 0.05$), however, from nine other treatments (Table 1). The LSD and SSN procedures agreed except that the SSN included the 'Golden Star' selfed seedling among the subset of treatments (11) which included the best treatment (smallest mean; SSN upper limit = 37.9). Sampling date effects were not significant ($P > 0.05$; LSD) for 'Arkin' or 'Fwang Tung' treatments.

The accessions 'Dah Pon' × open pollinated seedlings 1 and 2, 'Golden Star' × 'Fwang Tung' seedlings 1, 2, 3, 4, and 6, 'Dah Pon' × 'Fwang Tung' seedling, 'Arkin', 'Demak', and 'Golden Star' selfed seedling warrant further host resistance screening. Further resistance screening may include methods proposed by Cowley et al. (1992), including laboratory and field oviposition trials (antixenosis), natural field infestation levels, and sampling for detection of flies where and when fruits are harvested. They may also include factoring of variability among trees, and factoring of variability among commercial cultivation methods. Cultivars or seedlings demonstrating high resistance to the Caribbean fruit fly may be promoted and grown in Florida for export. If those identified as highly resistant are not commercially valuable, attributes conferring resistance in them could be identified and introduced into commercial cultivars through breeding or other methods.

SUMMARY

Resistance in carambolas to the immature stages of the Caribbean fruit fly was demonstrated. Cultivars or seedlings demonstrating resistance should be considered for promotion and production in Florida.

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EFFECT OF A PARASITIC WATER MITE ON THE PER CAPITA RATE OF INCREASE OF ITS HOST *HYDROMETRA AUSTRALIS* (HEMIPTERA: HYDROMETRIDAE)

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In some geographic regions within its range, the surface-dwelling aquatic insect *Hydrometra australis* Say (Hemiptera:Hydrometridae), formerly known as *Hydrometra myrae* Bueno, is commonly parasitized by larvae of the water mite *Hydryphantes tenuabilis* Marshall (Acari:Hydryphantidae), a possible synonym of *Hydryphantes ramosus* Daday (Lanciani 1971). Although mites initially attach to nymphal as well as adult hosts, those attaching to nymphs feed little if at all. Instead they remain attached to the host as it develops to the adult stage, at which time the mites begin to engorge. Within 7 to 13 days of attaching to adult hosts, the mite larvae complete feeding, metamorphose to a nonparasitic stage, and detach from the host.

The relatively large size attained by parasites during the brief engorgement period (Lanciani 1975a) suggests that hosts suffer some consequences of parasitism, and significant parasite-induced reductions in survival and age-specific reproductive rates have been reported (Lanciani 1975b). Unfortunately, the parasitic effect on the most critical and inclusive measure of host fitness, the per capita rate of increase (r), was not determined then because methods of estimating variance in r were not readily available. However, estimating variance in r is relatively routine now because of modern statistical methods and computer accessibility, thus recognition of significant differences among sample r values is possible. Accordingly, in the present study I

TABLE 1. GROUP ESTIMATES OF r, JACKKNIFE ESTIMATES OF r, AND 95% CONFIDENCE INTERVALS OF EACH JACKKNIFE ESTIMATE OF r IN 4 GROUPS OF *H. AUSTRALIS* (N = 20) BEARING 0, 2, 6, AND 10 MITES PER HOST.

Parasite Load	Group r	Jackknife Estimate of r
0	0.080	0.080
		(0.075 - 0.085)
2	0.072	0.071
		(0.066 - 0.076)
6	0.059	0.059
		(0.051 - 0.067)
10	0.048	0.049
		(0.036 - 0.062)

re-evaluate life-table data from Lanciani (1975b), comparing r values of *H. australis* host groups harboring different loads of the water mite *H. tenuabilis*.

Details of the laboratory procedure were as described in Lanciani (1975b). Adult female hosts, the stage and sex focussed on in this study, were held individually in plastic petri plates lined with moistened absorbent paper. An adult male was kept with each female for 3 to 4 hours each day to ensure that females mated. Plates were placed in groups of 12 into covered clear plastic boxes, which were kept in constant-temperature chambers maintained at 28° C and a 12:12 (L:D) cycle. Hosts were fed male *Drosophila melanogaster* Meigen that had been frozen. Male flies of only the first generation produced by a fly culture were used because of greater body-weight constancy. In the original experiments, food levels of 1/2 (i.e., 1 fly every other day), 1, 2, and 3 flies per day were used, but data from only the lowest food level were included in the present study because all food levels produced similar results and the much greater life span at the lowest food level yielded a longer oviposition period.

Mite loads were controlled by manipulating mite numbers on fifth instars so that adult females emerged with 1 of 4 parasite loads: 0, 2, 6, and 10 mites per host. Each parasite-load group contained 20 host individuals.

Survivorship, l_x, and age-specific fecundity, m_x, were determined on adult females in each parasite-load group from daily survival and reproductive records. A sex ratio of 1 was assumed, so m_x, which refers only to female eggs, was based on 1/2 the actual number of eggs laid per day. The per capita rate of increase, r, was found by trial and error substitution in the Euler-Lotka equation: $1 = \Sigma e^{-rx} l_x m_x$.

Variance in r was determined with the jackknife method (Tukey 1958), following the procedure outlined by Krebs (1989). This method seems to be as effective in estimating variance in r as the bootstrap method (Meyer et al. 1986). Knowledge of this variance then permitted (1) estimation of confidence intervals and (2) tests of significance with the Tukey method of multiple comparisons (Meyer et al. 1986).

Group estimates of r (i.e., the r of the entire cohort of 20 individuals in each parasite-load group), jackknife estimates of r, and 95% confidence intervals of each jackknife estimate were determined (Table 1). In each case, the jackknife estimate of r was within 0.001 of the group r. Also, r decreased relatively uniformly as mite load increased. The r of unparasitized hosts was significantly greater ($P < 0.05$) than that of hosts parasitized by 6 or 10 but not 2 mites.

The drain on internal resources of *H. australis* by *H. tenuabilis* reduces the insect's reproduction and survival (Lanciani 1975b). Because of the mathematical relationship between r and reproduction and survival rates described by the Euler-Lotka equation, the parasite would be predicted to reduce host r. This prediction was realized in the present study. Thus, parasitized populations of *H. australis* would increase slower than would unparasitized populations. The effect on host r is especially strong because the mite exploits the host in early adult life when reproduction is otherwise maximal. Many other species of parasitic water mites adversely affect host life-history parameters related to fitness (Smith 1988). Thus, reductions in r values of other water-mite parasitized hosts are likely to occur also.

Summary

The effect of the parasitic water mite *Hydryphantes tenuabilis* on the per capita rate of increase (r) of the host insect *Hydrometra australis* was determined using the jackknife method of estimating variance in r. The mite, which is known to reduce survival and age-specific reproductive rates, reduced host r as well; r was significantly higher in unparasitized hosts than in hosts parasitized by 6 or 10 mites.

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MORPHOLOGICAL EVIDENCE FOR POSSIBLE SITES OF PRODUCTION OF THE FEMALE SEX PHEROMONE OF *COPITARSIA CONSUETA* (LEPIDOPTERA: NOCTUIDAE)

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Copitarsia consueta (Walker) is a polyphagous insect that could be a serious pest of several cultivated plants in Mexico (Gutierrez & MacGregor 1983). Recently, some aspects of reproductive behavior were studied (Rojas & Cibrian 1994). Crude extracts of the female terminal abdominal segments tested in a wind tunnel evoked in males the following behavioral acts: oriented flight, landing, wing-fanning, spreading genitalia and attempted copulation (Rojas et al. 1993). Until now, the exact location of the sex pheromone gland remained unknown. This paper provides morphological and histological evidence of putative cells involved in the production of sex pheromone of *C. consueta*.

The insects used in this study were reared at 25 ± 2 °C, 65 ± 5% RH, and 14:10 (L:D) photoperiod regimen on an artificial diet (Rojas et al. 1993). Virgin females 4-6 days old were anesthetized with CO_2, the terminal portions of their abdomen extruded to a normal degree by gentle squeezing, and the abdomen ligated to keep the last segments extruded. The abdominal tips were cut off anterior to the ligation and fixed in Duboscq-Brasil (Pantin 1946) for 72 h. For scanning electron microscopy, the material was dehydrated in ethanol and critical-point dried. The tissue was mounted on metallic cylindrical stages, coated with gold and examined with a JEOL 35-C scanning electron microscope, operated at 10 Kv. For histological observations, the tissue was dehydrated in ethanol and embedded in paraffin. Serial, longitudinal and transverse sections were made at 10 µm, stained with hematoxylin-erythrosin and mounted in Canada balsam. Photomicrographs were taken with a Zeiss photomicroscope.

Analysis of the integument of the last abdominal segments suggests that two types of structures are involved in the production and release of pheromonal components. The first involves intersegmental membranes VII-VIII and VIII-IX (Fig. 1, No. 1,2) which reveal a characteristic histological structure on which the external surfaces are covered with a multitude of sharp epicuticular projections (Fig. 1, No. 4). These epicuticular projections rest over a stratum of flexible procuticle. Possible glandular cells present on this integument are similar to those found in other noctuids (Percy-Cunningham & MacDonald 1987). The pheromone producing glands in other Noctuidae are located in intersegmental membrane VIII-IX (Percy-Cunningham & MacDonald 1987). No prior study has found pheromone-producing cells in intersegmental membrane VII-VIII in this family. However, in the butterfly *Argynnis addippe*, the gland is located over the intersegmental membrane of abdominal segments VII-VIII (Percy & Weatherston 1971).

The second structures involved in pheromone production are the two lateral cuticular sacs situated in intersegmental membrane VII-VIII (Fig. 1, No. 3). At this place the integument is invaginated at each side to form an almost spherical cavity of extremely thick cuticle. It is formed by a thick layer of lamellated procuticle and a thin layer of superficial epicuticle (Fig. 1, No. 5,6). The hypodermis of this integument is

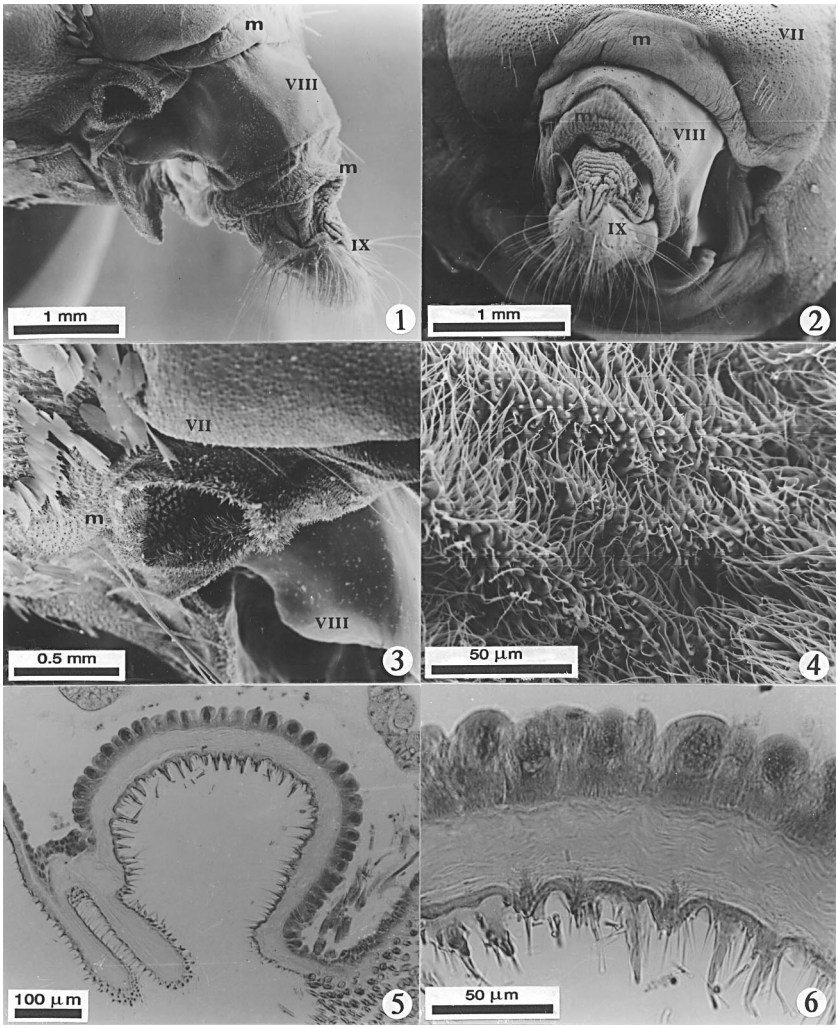

Fig. 1. Electron micrograph of possible site of production of female sex pheromone of *Copitarsia consueta*. **No. 1**, left lateral view of the abdominal segments everted during the calling behavior; m, intersegmental membrane; VIII, eighth abdominal segment; IX, ninth abdominal segment. **No. 2**. Dorsal view of the female abdominal terminalia; m, intersegmental membrane; VII, seventh abdominal segment; VIII, eighth abdominal segment; IX, ninth abdominal segment. **No. 3**. Aperture of the lateral glandular sacs in the intersegmental membrane VII-VIII; m, intersegmental membrane; VII, seventh abdominal segment; VIII, eighth abdominal segment. **No. 4**. Microtrichia covering the membranes VII-VIII and VIII-IX involved in the release of pheromonal components. **No. 5**. Longitudinal section of the tegumentary glandular sacs. **No. 6**. Section of the cuticle and hypodermis of the lateral tegumentary sacs.

formed by globe-shaped cells with nuclei that occupy approximately 70% of their volume (Fig. 1, No. 6). These cells exhibit typical characteristics of glandular epithelium, such as cellular membrane infolding along the whole line between cell and cuticle. In a light microscope, the cytoplasm has a fibrous appearance because it is always observed in cells with deep basal folds of the cellular membrane and in cells loaded with endoplasmic reticulum. These characteristics are typical of cells with intense synthesis activity. The nuclei are granulate and show a clear nucleolus. The cuticular structures continue beyond the borders of the orifices (Fig. 1, No. 3) with structures of the intersegmental membrane already described. The histological structure of the cuticular sacs suggests that these semispherical cells could synthetize the sex pheromone of this species, which would then have to diffuse through the cuticle to reach the cavity of the organ. This type of cell could be classified as class 1 (Noirot & Quennedey 1974). The females of many Lepidoptera produce their sex pheromone from glands of this type (Noroit & Quennedey 1974). The lateral cuticular sacs located in intersegmental membrane VII-VIII have not been reported in any species of any family. In *Bombyx mori*, the gland is represented by ventrolateral sacs in membrane VII-VIII (Hecker & Butenandt 1984).

The fact that in *C. consueta* we found different regions involved in the possible biosynthesis of pheromones is not new in the literature, since the same phenomenon is found in other species (Chow et al. 1976; Teal et al. 1983). In *Heliothis virescens*, it is suggested that different areas could produce or release mixtures of several pheromonal components (Teal et al. 1983).

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Summary

This paper provides morphological and histological evidence of the putative cells involved in production of the sex pheromone of *Copitarsia consueta* (Lepidoptera: Noctuidae). The analysis of the integument of the last abdominal segments suggests two types of structures may be involved: (1) the intersegmental membranes VII-VIII and VIII-IX, and (2) the two lateral cuticular sacs situated in intersegmental membrane VII-VIII.

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A REVISION TO THE BIBLIOGRAPHY OF THE SUGARCANE ROOTSTALK BORER WEEVIL, *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

FLORIDA *DIAPREPES* TASK FORCE, RESEARCH COMMITTEE[1]

DAVID G. HALL, CHAIRMAN

During the past 30 years, the West Indian sugarcane rootstock borer weevil, *Diaprepes abbreviatus* (L.), has spread from its original site of introduction near Apopka, FL to 15 counties throughout the citrus industry. This native pest of the Lesser Antilles of the Caribbean region is considered a major long-term threat to Florida agriculture. Nineteen species of *Diaprepes* are prevalent in nearby countries within the Caribbean region (Woodruff 1985), but Florida is currently the only state within the U.S.A. with the weevil. In addition, some species within the closely-related genus *Exophthalmus* are major pests of citrus and other agricultural crops in many Caribbean countries excluding Florida.

The biology of *D. abbreviatus* is typical of many curculionid weevils. The adults emerge from the soil, feed, and oviposit on a preferred host plant. As the eggs hatch, larvae fall to the soil surface where they burrow into soil to feed on the roots of the plant. After many months, the larvae pupate in the soil. The adults emerge after a few weeks, leave the soil again and start a life cycle that is variable, ranging from 8 to 24 months. The adults feed on the foliage of at least 41 plant species in Puerto Rico (Martorell 1945) whereas the larvae also appear to be polyphagous with a particular affinity to bore into the plant roots. Host plants such as sugarcane, yams, pineapple, and corn exhibit this type of injury. On the other hand, citrus appears to be a preferred host, but injury is characterized by the destruction of fibrous roots and the cortical layer of both lateral and crown roots.

About 50,000 acres of citrus in Florida have confirmed infestations of the weevil. Most of the infested acreage is exhibiting severe decline or is out of production. Within the last 4 years, *Diaprepes* has been detected in 7 new counties including more recent plantings in Collier, Hendry, and Glades counties completing its dispersal to all major citrus growing areas of the state. Adult weevils discovered in the Moore Haven area in an ornamental nursery in 1993 place the pest within the northwestern edge of the sugarcane growing area in Glades County. *D. abbreviatus* is also a serious threat to the foliage and ornamental plant industry of Florida. Currently, 94 commercial plant nurseries are known to be infested throughout Florida. Nurseries have strict quarantine regulations placed upon them when adult and/or larvae are detected on ornamental plants. Most seriously, recertification is virtually impossible since no effective methods are available to assure weevil eradication.

Because of the increased concern over the spread of *D. abbreviatus* and its devastating effect on the citrus tree in Florida during the past years, a grower-initiated Task Force was organized under the leadership of Mr. J. B. Pratt, Polk County citrus grower, and Ms. Connie Riehard of the Florida Department of Agriculture and Consumer Services. This 27 member Task Force has as primary objectives: 1) establishment of a grower awareness program within the citrus and ornamental industries to

[1]Members of research committee: Robert Adair, James Griffith, David Hall, Walter Kender, Richard Mayer, Clayton McCoy, Herbert Nigg, J. B. (Buster) Pratt, William Schroeder, Jeffery Shapiro, Jim Simpson, Sam Simpson, Omelia Sosa, John Taylor, and Gary Tucker.

combat the spread of *Diaprepes* and 2) encourage both short- and long-term strategies for control of this pest through research and extension programs.

In view of the fact that *D. abbreviatus* is becoming an increasingly important economic pest in Florida, threatens agriculture in other states and current biological and chemical control methods are limited, the Task Force agreed to update the bibliography published by Beavers et al. 1979 to assist scientists in their pursuit of a solution to this problem. Bibliographic sources were generated by both federal and state scientists involved in weevil research through various library services and include technical reports, popular articles, etc. that may be non-referred, but important as a source of new ideas.

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BOOK REVIEWS

HAWKINS, B. A. 1994. Pattern and Process in Host-Parasitoid Interactions. Cambridge Univ. Press, Cambridge. x + 190 p. ISBN 0-521-46029-8. Hardback. $42.95.

In 1984, when I was at the University of the West Indies, Trinidad, it was intriguing to find that the headquarters of the Commonwealth Institute of Biological Control was situated only a mile or two away. CIBC had long been involved in biological control programs around the world, introducing predators and parasites against pest insects. Two large catalogues produced by CIBC, the Herting Catalogue and the Thompson Catalogue, had been developed to assist biological control workers. The catalogues had extensive listings of host insects and their recorded parasites. I felt sure that there was a useful publication in these catalogues somewhere, and I summarized the data to see whether major insect orders, such as Lepidoptera, Diptera, Coleoptera and Hymenoptera, differed in the number of parasite species attacking them. I remember that larval Lepidoptera had many more recorded parasitoids than other orders. Unfortunately, my manuscript was rejected.

In 1987, I was pleased to see a paper by Brad Hawkins and John Lawton entitled "Species richness for parasitoids of British phytophagous insects," which used the **primary** literature to assess numbers of species of parasitoids associated with various types of insect hosts. An analogy can be found in the work of Southwood, Strong, Lawton and others who have analyzed the difference in the numbers of insect species associated with various plant species. In this earlier work, area proved to have a large effect: more widespread plants accrued greater numbers of insect species. Other factors, such as plant architecture—trees versus bushes versus grasses—also had an effect. Hawkins' new book essentially takes this concept to the next trophic level. If more-widespread plants have more species of insects, what controls the number of species of parasites on those insects? Unfortunately, as Hawkins points out, a similar analysis using the area of distribution of insects and number of parasites they support is not yet possible. No comparable atlases which detail the distribution patterns of insect species currently exist. Instead, Hawkins investigates the affects of seven "general types of independent variables," host feeding niche, host taxon, host generic diversity, sample size, climate, host food plant type and habitat type on parasitoid richness. The effects of these variables are determined on eight dependent variables: primary parasitoid species richness; parasitoid species richness by taxon, by biology (idiobiont/koinobiont), and by host stage attacked; parasitoid relative abundance; hyperparasitoid species richness; parasitism rate (the maximum total apparent parasitism rate reported for any single host population and for generation); and biological control success rate (the proportion of parasitoid introductions which have resulted in some level of pest control). Obviously there are problems in scoring some of these variables—when is biological control a success; how does one accurately assess parasitism rate when so many workers have documented variation in parasitism rates with density—but Hawkins does an admirable job of discussing most of these problems "up front."

What does Hawkins conclude? There are so many patterns and so many explanations that a listing of them here would be too extensive. However, one of Hawkins' main conclusions is that many observed patterns are consistent with a "susceptibly hypothesis"—host refuges from parasitism form the mechanistic basis for main relationships observed. Bottom-up effects are important—the biological attributes of plants and insects affect the size and species composition of parasitoid communities.

Within these broad generalizations readers will find many details to stimulate further thought.

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HILL, D. S. 1994. Agricultural Entomology. Timber Press; Portland, Oregon. Hardback, 640 p., 27 × 20 cm. ISBN 0-88192-223-4. $89.95.

Dennis Hill is well known for his authorship of two previous volumes on agricultural pests of the tropics and of temperate regions. In these he provided a world-wide perspective on insect pests, providing brief accounts of their biology, damage, and distribution. He also included useful lists of host plant associations and information on pest control.

In this new treatise, which is being marketed as a college textbook, Dennis Hill takes a decidedly different approach to the presentation of information while preserving the international flavor of his works. *Agricultural Entomology* takes a taxonomic approach to classification of pests rather than the more traditional grouping of pests by crop or geographic region. He reviews all major orders, the important families, and selected subfamilies, providing general information on their morphology and habits. Included in each section is a list of the most important species, accompanied by a few key facts such as host range and distribution; this usually comprises only a line or so of information. With over 300 photographs and 400 drawings, this text is unusually well illustrated.

The strengths of this book include the large format, copious illustrations, and world-wide perspective. The paper quality, format, and binding seem to be an improvement over the aforementioned agricultural pest volumes, thereby making it more suitable for student use. The introductory material, 100 pages in length, make a good introduction to insects, their biology, interrelationships with the environment, and the basis for "pesthood."

Weaknesses include the questionable value of long lists of pest species with only cryptic information about them, the lack of **any** information on pest control, and the overly brief description of collection, preservation, and identification. No keys are included. The only references recommended for collection and preservation techniques are British. The pests selected for inclusion seem, perhaps not surprisingly given Hill's experiences, to slight the western hemisphere. While the author has obviously made an effort to include some pests that are exclusively American, I anticipate that students will be unhappy about a book that pictures so few collectible insects.

There are, of course, some spelling errors and mislabeled pictures; it seems impossible to publish without a few. On the whole, however, the book is rather accurate. The biggest source of irritation for both students and professors will be the use of common names. Every continent (sometimes each country) seems to have its own set of common names, and many that are included in this book will not be recognizable to American users. An example that will plague readers of this book is *Diabrotica* spp. Hill's figure 10.97 is called southern corn rootworm but it is (and was in the "pests of temperate regions" book) the northern corn rootworm. The scientific name ascribed to this figure matches neither common name according to current use patterns.

There is some value in having pests arrayed taxonomically—it reinforces the importance of identification and reduces the duplication that occurs when polyphagous

insects are associated with several crops. For this approach to work with students, however, previous exposure to a course that includes identification will be needed. Alternatively, supplementary keys could be provided. I can't imagine an agricultural entomology course without information on pest control, so some supplementary information is likely needed here also. Looking past these annoyances, I think that on the whole Hill has made a useful contribution to entomology education through the publication of this book. Not only does he provide instructors with an alternative organizational approach, nicely complementing the current books on the market, but he provides a world-wide perspective that is increasingly valuable in our more mobile and international society.

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DEYRUP, M., AND R. FRANZ (eds.) 1994. Rare and Endangered Biota of Florida. Vol. IV. Invertebrates. University Press of Florida; Gainesville, xxx + 798 p. ISBN-0-8130-1323-2. Paperback. $39.95 (and ISBN-8130-1322-4. Hardback. $75.00).

This is the second, revised edition of a book published in 1982. The original edition was 19.5 × 26.6 cm with 131 pages, 86 of them on insects, and was vol. VI of the series. The original series was: I-Mammals, II-Birds, III-Amphibians and Reptiles, IV-Fishes, V-Plants, VI-Invertebrates, and VII-Liaison and Recommendations. How the original vol. VI became vol. IV of the new series is not explained. I suspect that catalogers will use the copyright date (1994) which is printed on the volume rather than the publication date (1 March 1995) which appears only separately in advertising material.

This new volume is 15 × 22.7 cm with 478 pages on insects and 30 on arachnids. The advertising material states that it "contains descriptions of 350 species (of at least 50,000 that exist in Florida) that the Florida Committee on Rare and Endangered Plants and Animals deem to be in decline or in danger of extinction." For most species mentioned there are five headings with brief text (DESCRIPTION, RANGE, HABITAT, LIFE HISTORY AND ECOLOGY, and BASIS FOR STATUS CLASSIFICATION). There also is, for most species, a map showing recorded distribution in Florida (by county) and the rest of the New World from the northern part of South America to the Canadian border. Habitus drawings are provided for some adults (Blattaria, Orthoptera, Scarabaeidae, Erotylidae, Formicidae and Mutillidae) and larvae (Trichoptera), whereas adults of Odonata and butterflies are illustrated by photographs, and other taxa are not illustrated. An English-language name has been coined for almost all of the species mentioned.

Insect taxa dealt with are Ephemeroptera (all), Odonata (all), Orthoptera (all), Blattaria (all), Mallophaga (all, but only as a list, without species-by-species treatment), Coleoptera (families Cicindelidae, Scarabaeidae, Cerambycidae, Histeridae, Cebrionidae and Erotylidae only), Trichoptera (all), Lepidoptera (Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae and Pieridae only), Diptera (Psychodidae, Culicidae, Chironomidae, Tabanidae, Mydidae, Empididae, Anthomyiidae and Asilidae only), Hymenoptera (Formicidae, Mutillidae, and Colletidae only). For these taxa, someone judged which were the species with most restricted distribution and/or the lowest population densities in Florida. For most, but not all taxa, all species were considered before judgment was made.

It may have been the hope of the producers of the series of volumes that this volume on invertebrates would begin to match in coverage that of the volumes on vertebrates and plants. Though the editors have expanded this volume greatly (cf. the first edition) any hope of effective coverage of all the rarest of the estimated 50,000 species of invertebrates cannot now be met because of the paucity of information. Whole phyla of invertebrates are not even mentioned. There are more pages about insects than about any other class, but even so, the proportion of insect families evaluated for rarity of their species is very low. The sad truth is that very little is known about Florida's rare invertebrates compared with its vertebrates.

Criteria for inclusion in the volumes of this series take into account only the Florida distribution. Thus, a species which is at the edge of its range in Florida (its major distribution may be in Appalachia or Cuba) and consequently is uncommon, is to be included; examples are the mosquitoes *Anopheles albimanus* Wiedemann and *Culex bahamensis* Dyar & Knab, and the butterflies *Eurema nise* (Cramer) and *Kricogonia lyside* (Godart). It is rational that invertebrate species and vertebrate species should be given equal status in these volumes. It would be especially interesting to prepare balance sheets of expenditure of funds to manage populations of *Anopheles albimanus* and *Felis concolor*, both of which are treated in the volumes as "endangered species", because both have caused loss of human life in parts of the Americas.

An enormous amount of basic research on insects and other invertebrate animals is needed before the successors of this volume truly represent Florida's rare and endangered invertebrate biota. This volume, to its credit, offers vignettes of some of Florida's rarer insects.

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