

EFFECT OF PHEROMONE BAIT STATIONS FOR SAP BEETLES  
(COLEOPTERA: NITIDULIDAE) ON *ANNONA* SPP. FRUIT SETJ. E. PEÑA<sup>1</sup>, A. CASTIÑEIRAS<sup>1</sup>, R. BARTELT<sup>2</sup> AND R. DUNCAN<sup>1</sup><sup>1</sup>University of Florida, Tropical Research and Education Center, 18905 SW 280 St.,  
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Atemoya, *Annona squamosa* L. x *Annona cherimola* Miller, and sugar apple, *A. squamosa*, have importance for the tropical fruit industry in Florida, but their yields are unreliable. This is so because fruit set is erratic due to highly variable pollination and physiological stress. Flowers of atemoyas and sugar apples initially undergo a female phase during which stigmas are receptive, and later they have a male phase when the anthers split to shed pollen, but the stigmas are no longer receptive (Gottsberger 1970). This prevents autogamy (i.e., fertilization of ovules by pollen from the same flower) (Nadel and Peña 1994). Most Annonaceae species are cantharophilous (beetle-pollinated), and a few are sapromyophilus (fly-pollinated) or thrips-pollinated (Gottsberger 1985). Cross pollination of *Annona* spp. can be carried out by sap beetles (Nitidulidae) (Reiss 1971, Nagel et al. 1989, George et al. 1992). In south Florida, nitidulid pollinators are in the genera *Carpophilus* (six species) and *Haptoncus* (one species) (Nadel and Peña 1994). Gottsberger (1985) suggested that the Annonaceae and other primitive plants with cantharophilous pollination had evolved a specialized pollination system by releasing heavy floral volatiles that attract certain beetle species. For example, atemoya flowers open mid- to late afternoon and allow beetles enter. The flowers begin to shed pollen around noon of the following day, and drop their petals by the following noon when the beetles start their departure. The petals emit an odor reminiscent of ripe, fermenting fruit that attracts the pollinators (Podoler et al. 1984). Current practices to manage pollinator populations in annona groves have resulted in inadequate yields (Schroeder 1971, George et al. 1992). Efforts to increase nitidulids in the flowers with fermenting pineapples as a bait have given mixed results (Galon et al. 1982, George et al. 1992).

Fruit setting in Annonaceae can be also be improved by hand pollination, but this is expensive and time consuming (Kahn and Arpaia 1990), or by improving attraction to the sap beetles by the use of lures (Nadel and Peña 1994). Several pheromones have been identified in the *Carpophilus* species complex (Okumura and Savage 1974) and tested in date groves in California (Bartelt et al. 1992, 1994). The effect of the pheromones is synergized when they are used in combination with food odors (Bartelt et al. 1992). In this study we evaluate the effect of sap beetle pheromones and food odor bait as attractants for beetle pollinators and their effect on fruit set of sugar apple and atemoya.

Synthetic hydrocarbon pheromones used in this study were available from previous research (Bartelt et al. 1995). The compounds were: (2E, 4E, 6E)-5-ethyl-3-methyl-2,4,6-nonatriene (1), (3E, 5E, 7E)-6-ethyl-4-methyl-3, 5, 7-decatriene (2), (3E, 5E, 7E)-5-ethyl-7-methyl-3,5,7-undecatriene (3), (2E, 4E, 6E, 8E)-3,5,7-trimethyl-2, 4, 6, 8-decatetraene (4), (2E, 4E, 6E, 8E)-3, 5, 7-trimethyl-2, 4, 6, 8-undecatetraene (5), (2E, 4E, 6E, 8E)-7-ethyl-3, 5-dimethyl-2, 4, 6, 8-decatetraene (6), (2E, 4E, 6E, 8E)-7-ethyl-3, 5-dimethyl-2, 4, 6, 8-undecatetraene (7) and (3E, 5E, 7E, 9E)-6, 8-diethyl-4-methyl-3, 5, 7, 9-dodecatetraene (8). Rubber septa were prepared with these compounds in the following percentages as baits for five *Carpophilus* species: *C. freemani*, 1(96%), 7 (4%); *C. mutilatus*, 2 (7%), 3 (93%); *C. hemipterus*, 4 (67%), 5 (21%), 6 (7%),

7 (5%); *C. lugubris*, 5 (9%), 7 (91%); and *C. dimidiatus* 8 (100%). Proportions were chosen so that emissions from septa would be as similar in composition as possible to those from beetles. For all species except *C. dimidiatus*, a total of 500 µg of all-E isomers was used in each bait. The pheromone for *C. dimidiatus* was used at the higher rate of 1.2 mg per septum because of the lower volatility of compound 8, the pheromone for the species. Fermented whole-wheat bread dough (a 4:1:2:0.01 mixture of whole-wheat flour, sugar, water and yeast) was used as the synergist in all cases (~ 15 ml per trap) (Bartelt et al. 1994).

The studies were conducted from 21 March to 17 May 1995 by placing beetle traps (Dowd et al. 1992) baited with pheromone septa and bread dough in the perimeters of a 0.46-ha sugar apple orchard in Homestead, Florida. Traps (n = 12) were hung from the trees at 1.2 m above the ground and placed at a distance of ca. 28 m apart. Traps were inspected weekly and trapped species identified.

Effect of Bait Stations on Fruit Set. The study consisted of two experiments, each having a complete block design. For experiment 1 (27 April, 1995 -7 July 1995), the study was conducted in the same orchard mentioned above. The treatments included the pheromones of *C. mutilatus*, *C. freemani*, *C. lugubris*, *C. hemipterus* and *C. dimidiatus*, each used in combination with the dough as co-attractant. In addition there was an unbaited control and a treatment containing just the bread dough. Flowers were recorded per tree and attractants were hung only in those trees with more than 100 tagged flowers. One bait station was hung per tree for 7 days, and then removed. Bait stations consisted of a wire holding a rubber septum with the pheromone, and a plastic cup with 15 g of bread dough. The baits were covered with a fabric sleeve to prevent contact by the beetles. Rows of 12 trees per treatment were used. Treatments were set 60 m apart.

For experiment 2 (September 12, 1996-October 11, 1996), the study was conducted in a 1-ha atemoya orchard in Homestead, Florida. The combinations of pheromones that gave the best results in experiment 1, plus dough, and the dough alone, were tested following the same procedure as explained above. From the second to the seventh week after treatment, percent fruit set per tree was calculated every week by dividing the number of fruits 1 cm in diameter or higher by the number of flowers tagged.

Percentages were transformed to arcsine of the square root of the proportion to stabilize variance, and these data were analyzed by using Statistical Analysis System general linear models (SAS 1987) for balanced ANOVA. Means were separated by Waller-Duncan k-ratio t-tests.

The dominant sap beetle species were *C. mutilatus* (32.9%), *C. freemani* (21.7%), and *C. dimidiatus* (19.4%), followed by *Colopterus* sp. (13.6%), *Haptoncus luteolus* (7.3%), *C. marginelus* (1.8%), *C. humeralis* (1.3%) and *C. hemipterus* (0.1%) (Table 1). One of these species, *C. mutilatus* was the most abundant species collected from atemoya flowers in Florida in 1988 followed by *H. luteolus*. (Nadel and Peña 1994). *C. mutilatus* responded best to the combination of its own pheromone plus fermenting whole wheat dough, and catches with this treatment were as high as 92 individuals in 1 wk in one trap. *C. mutilatus* responded less strongly to pheromones of other beetles. *C. dimidiatus* responded to its own pheromone and to the combination of its own pheromone plus fermenting whole wheat dough. *C. hemipterus* responded only to the combination of its own pheromone plus fermenting dough. *H. luteolus*, for which a pheromone is not known, responded consistently to the combination of the pheromone of *C. lugubris* plus whole wheat dough.

Percent fruit set was higher in 1995 than in 1996 (Tables 2 and 3). In 1995, the highest percent fruit was obtained for the combination *C. dimidiatus* plus dough com-

TABLE 1. MEAN TRAP CATCHES FOR 8 NITIDULID SPECIES DURING A SURVEY IN A SUGAR APPLE FIELD IN HOMESTEAD, FL FROM MARCH 21 TO MAY 17, 1995.

Attractant or Pheromone	Beetles per week per trap (mean)							
	<i>C. mutilatus</i>	<i>C. freemani</i>	<i>C. dimidiatus</i>	<i>C. marginelus</i>	<i>C. humeralis</i>	<i>C. hemipterus</i>	<i>H. luteolus</i>	<i>Colopterus sp.</i>
Dough	0.22 b	0.00 b	0.00 b	0.00 b	0.10 a	0.00 a	1.72 ab	0.00 c
<i>Cm</i> phero- mone, + Dough	19.70 a	0.80 b	0.00 b	0.00 b	0.00 a	0.00 a	0.60 ab	0.20 bc
<i>Cf</i> , phero- mone + Dough	1.20 b	23.70 a	0.00 b	0.40 ab	0.20 a	0.00 a	0.20 b	1.20 bc
<i>Cl</i> , phero- mone + Dough	1.60 b	0.60 b	0.00 b	0.50 ab	0.40 a	0.00 a	2.10 a	2.80 b
<i>Ch</i> , phero- mone + Dough	5.11 b	0.55 b	0.00 b	0.70 a	0.10 a	0.10 a	0.89 ab	8.00 a
<i>Cd</i> , pheromone + Dough	0.40 b	0.00 b	10.50 a	0.00 b	0.30 a	0.00 a	1.00 ab	0.10 bc

<sup>1</sup>*Cm* = *Carpophilus mutilatus*; *Cf* = *C. freemani*; *Cl* = *C. lugubris*; *Ch* = *C. hemipterus*; *Cd* = *C. dimidiatus*.

<sup>2</sup>The square root transformation  $x' = (x + 0.5)$  was applied to normalize the means. Original means are shown. Means in the same column followed by the same letter are not significantly different according to a Waller-Duncan k-ratio t-test ( $P > 0.05$ ).

pared to the percent fruit set in untreated trees or that from baited with *C. freemani* and *C. mutilatus* pheromones. Moreover, percent fruit set was low when *C. dimidiatus* pheromone was used without food bait. Bartelt (1992) observed that the effect of the pheromones was synergized by food odors and recommended the use of both attractants in combination. In 1996, more fruit was observed on those trees baited with either *C. dimidiatus*, *C. freemani* or *C. mutilatus* pheromones in combination with dough than on untreated trees or those trees baited with dough alone. In both years fruit retention decreased to 5-6% during the last 5-7 weeks after treatment, when the fruit reached more than 2 cm in diameter. The lower fruit retention found on weeks 5-7 after treatment might have been caused by water or fertilizer stress. It is well known that water stress and lack of nutrients induce fruit abscission (Salisbury and Ross 1978).

Previously, Nagel et al. (1989) demonstrated that fruit set of atemoya caged trees supplied with nitidulids was significantly higher (21%) than on caged trees without nitidulids (5.1%). Nitidulids were found to pollinate 10% of sugar apple flowers, and 29% of atemoya flowers in Florida (Nadel and Peña 1994). Our study suggests strongly that nitidulids increased pollination and percent fruit set in both sugar apple and atemoya, because significant differences between the treated and non-treated

TABLE 2. PERCENT FRUIT SET IN SUGAR APPLE TREES SEVEN WEEKS AFTER TREATMENT WITH PHEROMONE BAIT STATIONS: 1995.

Pheromone source <sup>1</sup>	% Fruit Set ± SE <sup>2</sup>
<i>Cd</i>	6.87 ± 1.00 c
<i>Cd</i> + dough	38.11 ± 5.10 a
<i>Cf</i>	11.06 ± 2.03 bc
<i>Cf</i> + dough	10.84 ± 5.00 bc
<i>Cm</i>	11.14 ± 5.21 bc
<i>Cm</i> + dough	10.36 ± 6.61 bc
Control (no bait)	4.33 ± 4.68 d

<sup>1</sup>*Cd* = *Carpophilus dimidiatus*; *Cf* = *C. freemani*; *Cm* = *C. mutilatus*.

<sup>2</sup>Means followed by the same letter are not significantly different according to a Waller-Duncan k-ratio t-test on arcsine transformed data ( $p > 0.001$ , k-ratio = 100). Untransformed means are presented.

TABLE 3. PERCENT FRUIT SET IN ATEMOYA TREES SEVEN WEEKS AFTER TREATMENT WITH PHEROMONE BAIT STATIONS: 1996.

Pheromone Source <sup>1</sup>	% Fruit Set ± SE <sup>2</sup>
<i>Cd</i> + dough	10.33 ± 3.39 a
<i>Cf</i> + dough	9.42 ± 4.37 a
<i>Cm</i> + dough	8.33 ± 4.78 a
Dough	4.33 ± 3.98 b
Control (no bait)	0.55 ± 1.24 c

<sup>1</sup>*Cd* = *Carpophilus dimidiatus*; *Cf* = *C. freemani*; *Cm* = *C. mutilatus*.

<sup>2</sup>Means followed by the same letter are not significantly different according to a Waller-Duncan k-ratio t-test on arcsine transformed data ( $p > 0.001$ , k-ratio = 100). Untransformed means are presented.

trees were observed each year (Tables 1 and 2). Our results show that pollination in annonas can be increased through the use of pheromone bait stations in South Florida. However, the bait stations should be used as part of an integrated management program including cultural practices designed to decrease physiological stress and improve fruit retention.

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## SUMMARY

The effect of nitidulid beetle (*Carpophilus dimidiatus*, *C. freemani* and *C. mutilatus*) pheromones, and combinations with food odor bait as attractants for pollinators were evaluated on fruit set of sugar apple and atemoya. More fruit was observed on those trees baited with either *C. dimidiatus*, *C. freemani* or *C. mutilatus* pheromones in combination with food odor bait than on untreated trees.

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