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TRAPPING OF *Phyllophaga elenans* WITH A FEMALE-PRODUCED PHEROMONE

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Abstract—Attraction of *Phyllophaga elenas* to vaned bucket traps baited with the recently identified female-produced pheromone, L-isoleucine methyl ester (LIME), is efficient. Pheromone-baited vaned buckets with water to retain insects were more effective than buckets without vanes or plastic containers with the sides cut out. Pheromone-baited vaned bucket traps from which water was omitted required the addition of a funnel below the vanes to retain insects. Normally used light traps were about 10 times more effective than pheromone-baited vane bucket traps in capturing *P. elenans*. Over 95% of *P. elenans* were captured between 6:00 and 9:00 PM. The male–female ratio was ~3–4:1 in both light and pheromone traps, and the ratio was relatively unchanged throughout the capture period. Most *P. elenans* were captured in the treed areas surrounding sugarcane fields. More *P. elenans* were captured in treed borders than in grassy borders of sugarcane fields. The effective radius of the pheromone-baited vaned bucket trap is between 5 and 15 m.

Key Words—*Phyllophaga elenans*, May and June beetles, pheromone trapping, sugarcane.

INTRODUCTION

Scarabaeidae in the genus *Phyllophaga* are commonly known as May or June beetles and have worldwide distribution (Westcott, 1964). In Central America, several *Phyllophaga* spp. are important pests of food crops (King, 1984). *Phyllophaga*

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larvae damage roots during their long life. *P. elenans* and *P. vincina* are economically important species from Guatemala to Costa Rica, and *P. elenans* is the most common destructive species in El Salvador and Honduras (King, 1984). In the dry tropical zone of northwestern Costa Rica, *P. elenans* is a major pest of several crops, the most important of which is sugarcane. This species is typical of other *Phyllophaga* spp. with a 2-year life cycle in which adults emerge just after moderate rains following annual dry periods (King, 1984). The major flight period is usually less than a month. During their short lives as adults, *P. elenans* emerge from the soil at dusk and fly to nearby plants that were either the crop that acted as a food source for larvae or other plants in the area. In related *Phyllophaga* spp., females emerge and fly first (King, 1984). Upon arrival at a suitable site, the female raises her abdomen, exposing the pheromone gland, and begins calling. Males arrive, and mating occurs. At daybreak, *P. elenans* return to the ground. A technique to manage the large populations of *P. elenans* that build up in sugarcane involves planting attractive trap trees, such as guacimo (*Guajunia ulmifolia*) and malinche (*Delonix regia*), around infested fields. Insecticide treatment of the trap trees during the flight season is used to kill adult *P. elenans*. Another commonly used technique is to trap adults with light traps.

Isoleucine methyl ester (LIME) has recently been reported as the female-produced pheromone for *P. anxia* by Zhang et al. (1997). Recent work (Leal et al., 2003) showed that LIME is the female-produced pheromone for *P. elenans*. In this work, we examined attraction of *P. elenans* to traps baited with LIME. We also examined the design and positional factors that affect the ability of pheromone-baited traps to capture *P. elenans*.

METHODS AND MATERIALS

Field Site. Experiments were carried out in the 3700-ha Central Azucarera Tempisque sugarcane plantation in the Guanacaste Province of Costa Rica. This is a dry tropical region in the northwest part of Costa Rica. The plantation has sugarcane of all ages with many plantings bordered by guacimo and malinche trees that are attractive food for adult *P. elenans* (King, 1984). Except for test 8, experiments were positioned in treed or grassy borders of sugarcane fields with intertrap distance of 10–50 m (Table 1). For test 8, each replicate (4 replicates/night) consisted of a set of equivalently baited traps in a line perpendicular to the border of a field and 30 m from adjacent replicates.

Traps. Trap A consisted of a 20-liter white plastic bucket containing 3–5 cm of detergent-laced (1–2%) water. Trap B was like trap A additionally topped by two intersecting 75-cm-high $\times$ 33-cm-wide vanes of galvanized metal. The vanes contained a 5-cm-diameter hole at the center of each vane to allow air to pass through the vanes freely. Pheromone lures were hung from wires so that their release area

TABLE 1. EXPERIMENTS

Test	Treatments	Date 1997	LIME (mg/day)	Trap Dist	SC Age Months	Statistics
1 (Fig. 2)	Trap A with no vane & trap B with different vane materials	May 2–5	2	35 m	2	3 Replicates/night, traps rerandomized nightly ANOVA ($N = 12$) log ($x + 0.5$) transformed pooled data $F = 4.72$, $df = 3, 42$, $P < 0.05$
2	Trap B vs D both LIME-baited and with galvanized metal vanes	May 26	8	35 m	9	ANOVA ($N = 5$), $F = 0.26$ $df = 1, 8$, $P \sim 0.662$
3	Trap B with galvanized metal vanes vs. container trap C	May 24	8	25 m	6	ANOVA ($N = 10$), $F = 38.62$ $df = 1, 18$, $P < 0.05$).
4	Trap B with white plastic vanes with 3–5 cm 3% detergent laced water vs. dry	May 6	11	50 m	2	ANOVA ($N = 8$), $F = 1.35$ $df = 1, 14$, $P \sim 0.263$
5	Trap B with white plastic vanes with cardboard funnel in bucket or 4 cm 3% detergent laced water in bucket vs. light trap (D)	May 14	8	35–50 m	9	ANOVA ($N = 8$), $F = 13.87$ $df = 2$, 22; $P < 0.05$
6 (Fig. 3)	Trap B with white plastic vanes with three different LIME release rates	May 2–3	2, 8, 11	35 m	2	Traps rerandomized nightly ANOVA log ($x + 1$) transformed pooled data ($N = 20$) $F = 38.12$, $df = 2, 55$, $P < 0.05$
7 (Fig. 4)	Trap B with white plastic vanes, 6 traps placed along treed border	May 14	8	10–20 m	2	Captured insects removed at 30-min intervals from 5:00 to 9:00 PM and 4:00 to 5:30 AM
8 (Fig. 5)	Trap B with white plastic vanes placed along border and in fields	May 4–5	8	30 m betw rep	8	Insects collected nightly, 4 replicates/night ANOVA ($N = 8$) square ($X + 1$) transformed pooled data $F = 11.95$, $P < 0.05$
9	Trap B with white plastic vanes placed along grassy or treed borders	May 18	8	50	9	ANOVA ($N = 9$), $F = 8.69$ $df = 1, 20$, $P < 0.05$).
10 (Fig. 6)	Trap B with galvanized metal vanes	May 23–24	8	Var	9	ANOVA ($N = 10$) 50-m vs. 15-m distance, NS, ANOVA on 50-m vs. 5-m dist, $F = 4.88$ $df = 1, 19$, $P < 0.05$.

was over these holes. Vanes protuded 20 cm into the bucket. Trap C consisted of a rectangular 20-liter plastic container with windows 16 × 23 cm or 20 × 23 cm cut into the sides. This trap contained 4 cm of detergent-laced (3%) water, and the pheromone lure was suspended from the top of the container near the center of the trap. Trap D consisted of two crossed 20-cm-wide × 35-cm-high galvanized metal vanes with a 10-cm open space at the center into which a portable (23-cm-long, 8-W, 12-V) florescent light was placed. Below the vanes was a galvanized metal funnel (50 cm diameter at top) that directed captured insects into a wire mesh basket (40 cm diameter × 80 cm deep) that retained insects. The entire assembly was mounted on a metal frame so that the light was approximately 1.3 m above the ground.

Lures. Pheromone lures were membrane release devices (ChemTica International, San Jose, Costa Rica) that released the described amounts of LIME under a daily temperature regime of $26 \pm 2^{\circ}$C maximum and $17 \pm 2^{\circ}$C minimum.

Statistics. Where experiments were conducted for more than one night, traps were rerandomized nightly and captures analyzed for date effect. In no case was a date effect detected, and capture data were pooled for comparison of treatments. Capture data were analyzed for normal distribution and, where necessary, transformed as indicated to achieve homogeniety. ANOVA (fully factorial routine) was conducted using Systat 5.2.1. Means are always presented untransformed and when topped or followed by different letters are significantly different by Bonferonni *t* test ($P > 0.95$).

RESULTS AND DISCUSSION

All experiments were conducted with LIME, identified as the female-produced pheromone of *P. elenans* (Leal et al., 2003). Initially, we examined the effect of trap design on capture rates of *P. elenans*. Several designs were examined (Figure 1). Bucket traps (A) were less efficient than bucket traps with vanes (B), and several vane materials gave statistically equivalent capture rates (Figure 2). During this experiment, we observed most *P. elenans* approached traps in the area of the buckets. Those that approached galvanized metal and white plastic vanes hovered near the vane and then dropped, while flying, into the traps. A higher proportion of insects that approached Plexiglas vanes flew into the vanes before dropping into the trap. Although traps with Plexiglas vanes captured numerically more insects, we did not observe statistical differences between capture rates of traps with different vane materials.

The bucket trap with galvanized metal vanes (B) is a variant of the vaned metal light trap (D) that is commonly used to capture *Phyllophaga* spp. We found that (test 2) LIME-baited vaned bucket traps (B) captured 40.8 ± 11.9 *P. elenans*/trap/night compared to 53.2 ± 21.0 for light traps (no light, D). Although LIME-baited light

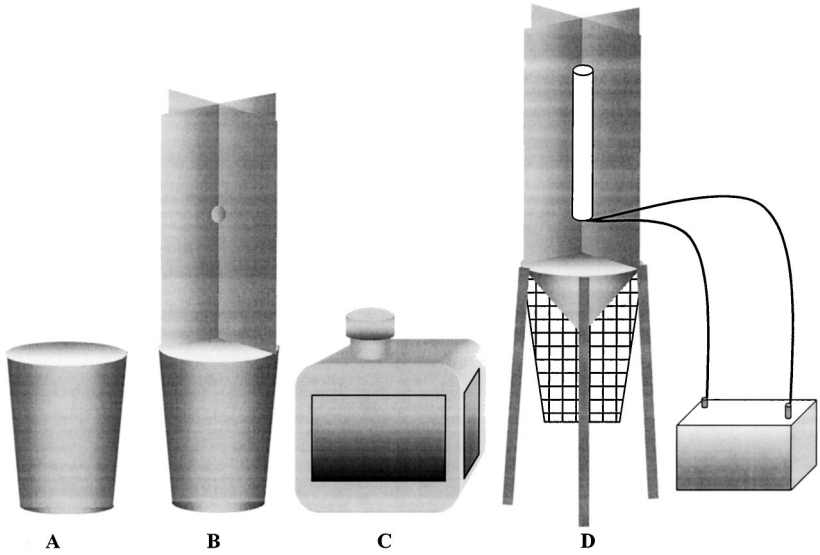

FIG. 1. Trap types examined for efficiency in capture of *P. elenans*.

traps captured numerically more *P. elenans*, no statistical difference was observed between the two vane trap designs.

Since *P. elenans* were observed to approach bucket traps with vanes in the area of the bucket tops, we examined the open bucket trap design that is readily available in the form of used insecticide containers. This design is based on 20-liter rectangular containers into which are cut large windows (C). We observed (test 3)

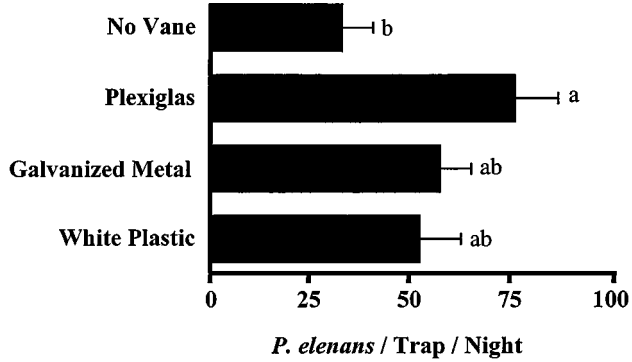

FIG. 2. Effect of no vane vs. different vane materials on *P. elenans* capture rates in bucket LIME-baited bucket traps (test 1). Bars followed by different letters are statistically significant at $P < 0.05$.

that LIME-baited vaned bucket traps (B) captured significantly more *P. elenans* (94.6 ± 6.0/trap/night) than container traps (C, 49.6 ± 4.0/trap/night).

The normal flight period of *P. elenans* spans the months of April, May, and June in the Guanacaste region of Costa Rica. This period is usually dry and windy but punctuated with periodic heavy rain. Since drowning is an easy way to retain captured insects, we compared (test 4) LIME-baited vaned bucket traps (B) that contained water with dry traps of the same design. We observed that traps containing detergent-laced water captured 942 ± 222.8 *P. elenans*/trap/night compared to 669.6 ± 70.0/trap/night for dry traps. Although traps containing water captured numerically more *P. elenans*, the differences were not statistically significant.

We attributed the above result to escape, and in a subsequent experiment (test 5), we examined the effect of adding a funnel under the vanes to retain captured insects. This test was conducted using LIME-baited vaned traps that had either a white cardboard funnel inserted into the bucket just below the vanes or detergent-laced water. In this test, the funnel-containing traps and the water-containing traps captured similar numbers of *P. elenans* (80.1 ± 27.6 and 84.7 ± 16.9 *P. elenans*/trap/night, respectively). In the same test, we found that these pheromone-baited vane traps (B) were significantly less efficient than light traps (D, 858.2 ± 176.7 *P. elenans*/trap/night). In a subsequent test, we determined that the release of LIME at the rate of 16 mg/day from light traps (D) numerically increased capture rates (∼65%) compared to unbaited light traps but that the increase was not statistically significant.

We examined the effect of LIME release rate on capture rate for bucket traps (B). We observed (test 6) that increasing LIME release rates between 2 and 11 mg/day increased capture rates (Figure 3). The observation that increasing pheromone release rate increases capture rates parallels observations for another economically important scarab, *Oryctes rhinoceros*. In the later case, increasing capture rates were found up to the highest release rate studied, 30 mg/day (Hallet et al., 1995). The above indicate that a bucket trap with white plastic vanes (B) with a lure releasing 11 mg/day of LIME is an efficient trap for *P. elenans*.

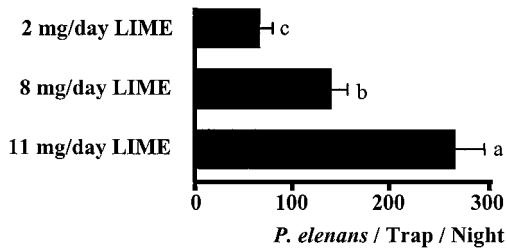

FIG. 3. Effect of different LIME release rates on *P. elenans* capture (test 6) in vaned bucket traps. Bars followed by different letters are statistically significant at $P < 0.05$.

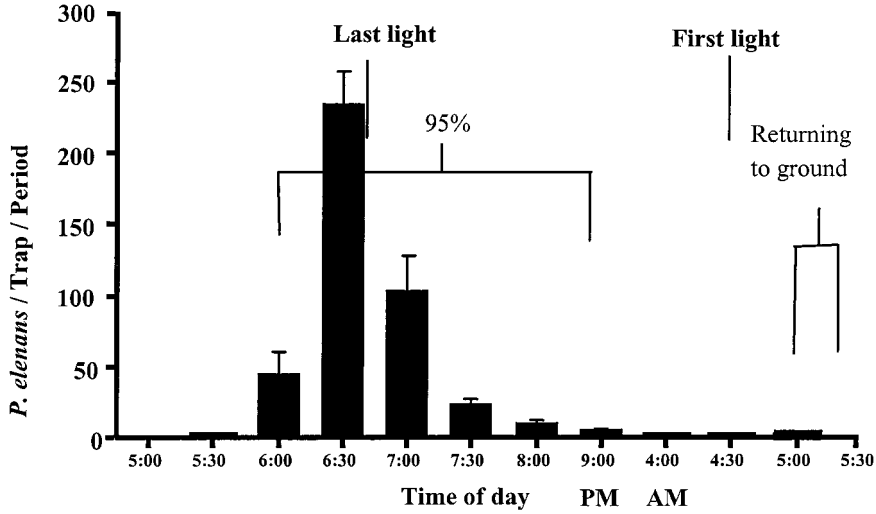

FIG. 4. Determination of time of day of flight of *P. elenans* (test 7).

Adult *P. elenans* are in the soil until sunset (~6:00 PM), at which time they fly within 2 hr to plants on which they feed and mate. At first light (4:30 AM), they become active again and begin to fly from feeding locations to the ground. This process is complete by 5:30 AM. The time-dependent response of *P. elenans* to LIME-baited bucket traps with white plastic vanes (B) was examined, and it revealed that 75% of all captures occurred between 6:00 and 7:00 PM and that this increased to 95% by 9:00 PM (Figure 4). During their return from feeding and mating sites to the ground, *P. elenans* are not attracted to LIME-baited traps.

The tendency of *P. elenans* to be more responsive to LIME-baited traps placed near food sources was observed in a comparison of capture rates of traps placed in the fields compared to traps placed at the borders of fields. Capture rates are significantly higher in traps along borders of fields containing trees than in the fields from which they emerge but in which they do not feed (Figure 5).

A further experiment (test 9) was conducted to determine if placement of traps on treed borders was more effective than placement on grassy borders. Traps in borders containing trees fed upon by *P. elenans* captured significantly more *P. elenans* (375.5 ± 53.2/trap/night) than traps in grassy borders (177.0 ± 23.6/trap/night).

During the flight season (late April to late May), we found the ratio of male to female *P. elenans* captured in LIME-baited vane bucket traps (B) to be rather constant at 4:1. This ratio was similar to that (3.3:1) obtained in light traps in the same sugarcane plantation during the same time period. Additionally, the sex ratio of *P. elenans* captured in LIME-baited traps within fields was the same as along borders. The similar sex ratio of *P. elenans* captured in LIME-baited and light

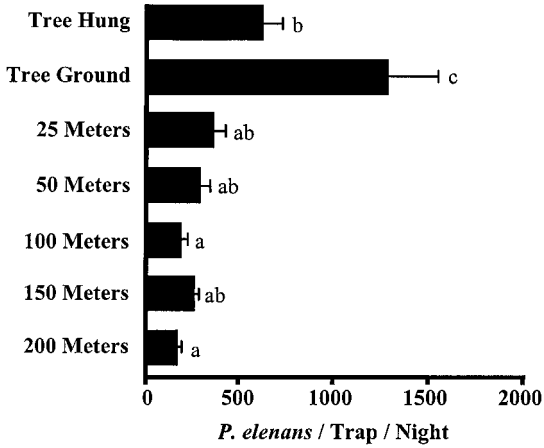

FIG. 5. Effect of trap location of capture rate of *P. elenans* (test 8). Traps (B) were placed under trees in which *P. elenans* feed and 25, 50, 100, 150, and 200 m from treed border of field. Bars followed by different letters are statistically significant at $P < 0.05$.

traps suggests LIME functions as an aggregation pheromone for *P. elenans*. In the case of *Oryctes rhinoceros*, male-produced ethy1-4-methyloctanoate attracts more females than males (Hallett et al., 1995). The ability of LIME to attract both male and female *P. elenans* increases its utility in reducing populations in mass trapping strategies.

Since it is likely that mass trapping *P. elenans* in mature sugarcane would involve placement of traps around the periphery of fields, we examined the effective radius of pheromone-baited traps in this configuration (Figure 6). Thus, we compared capture rates of traps in the center of an array of three traps in which the distances between the center and distal traps were 5, 15, or 50 m. There was no statistical difference in capture rates between traps possessing adjacent traps 15 or 50 m away, but when traps possessing adjacent traps 50 m apart were compared with those possessing adjacent traps 5 m away, the latter captured significantly fewer *P. elenans* (Figure 6).

This work has resulted in the development of an efficient pheromone-baited trap for *P. elenans* that is more economical than a light trap in cost per beetle captured. Light traps cost USD200, while vane traps with pheromone lures cost $\approx$USD10. Although light traps are $10\times$ more efficient than pheromone traps, they require the nightly servicing of battery retrieval to prevent theft. Thus, pheromone traps seem more economical. Since the completion of this work, major sugarcane plantations in Guancaste province of Costa Rica have adopted the strategy of trapping adult *P. elenans* during May and June. To date no data are available relating to the effect of trapping *P. elenans* on damage to sugarcane.

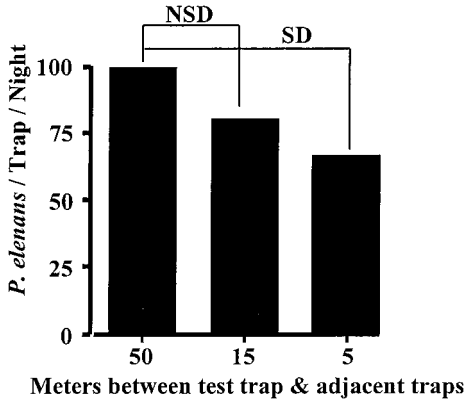

FIG. 6. Effect of proximity of adjacent traps on capture of *P. elenans* in LIME-baited vaned bucket traps (test 10). Traps were placed at 50-, 15-, or 5-m intervals along a road at the edge of 6-month-old sugarcane. On the first day, traps having adjacent traps 50 and 15 m away were compared, while on the second day traps having adjacent traps 50 and 5 m away were compared. Captures for both tests are scaled so that captures in traps with adjacent traps 50 m away equal 100 for both tests. SD = significant at $P < 0.05$, NSD = no significant differences.

One can imagine the use of a modification of this trap to allow use in pathogen deliverly. A combination of pheromone-based mass trapping, *Metarhizium anisopliae*, and baculovirus delivery has been shown to be an effective management system for high populations of *Oryctes rhinoceros* in which pheromone trapping alone is not sufficient to lower populations to economically acceptable levels (Tuck, 1996).

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REFERENCES

HALLETT, R. H., PEREZ, A. L., GRIES, G., GRIES, R., PIERCE, H. D., JR., YUE, J., OEHLSCHLAGER, A. C., GONZALEZ, L. M., and BORDEN, J. H. 1995. Aggregation pheromone of the Coconut Rhinoceros Beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae), *J. Chem. Ecol.* 21:1549–1570.

KING, A. B. S. 1984. Biology and identification of white grubs (*Phyllophaga*) of economic importance in Central America. *Trop. Pest Manage.* 30:36–50.

LEAL, W. S., OEHLSCHLAGER, A. C., ZARBIN, P. H. G., HIDALGO, E., SHANNON, P. J., MURATA, Y., GONZALEZ, L. M., ANDRADE, R., and M. ONO, M. 2003. Sex pheromone of the Scrab Beetle *Phyllophaga elenans* and some intriguing minor components, *J. Chem. Ecol.* 29:15–26.

TUCK, H. C. 1996. The integrated management of *Oryctes rhinoceros* (L) populations in the zero burning environment. Plam Oil Research Institute of Malaysia. *International Conference Proceedings, Kuala Lumpur*, September, A28:336–368.

WESTCOTT, C. 1964. The Gardeners Bug Book, 3rd ed. Doubleday & Co., New York.

ZHANG, A., ROBBINS, P.S., LEAL, W. S., LINN, C. E., JR., VILLANI, M. G., and ROELOFS, W. L. 1997. Essential amino acid methyl esters: Major sex pheromone components of the cranberry white grub, *Phyllophaga anxia*, (Coleoptera: Scarabaeidae). *J. Chem. Ecol.* 23:231–245.