OLFACTORY ATTRACTION OF THE SUGAR CANE WEEVIL (COLEOPTERA: CURCULIONIDAE) TO HOST PLANT ODORS, AND ITS AGGREGATION PHEROMONE.

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Abstract

This study provides a field test of the behavioral activity of the sugar cane weevil *Metamasius hemipterus* (L.) pheromone with the odors of its host plants, and describes the development of a laboratory two-cup pitfall olfactometer and a field trap to determine the effectiveness of baits. The aromatic sources were host plant odors: a) pseudostem and rhizome of the *Musa* sp "apple" banana (AAB) and b) stalks of sugar cane, *Saccharum* sp. The olfatometric results show that the host plant odors do attract the insects. Sugar cane is more attractive than banana rhizome, and pseudostem is as attractive as sugar cane stalk. Two field traps were tested, one with a lid and the other with a funnel. The capture efficiency of the two traps was evaluated at two heights (0 and 1 meter) and with aggregation pheromone alone, pheromone plus banana pseudostem, pheromone plus sugar cane, pheromone plus banana rhizome, and pheromone plus pineapple as baits. The results indicate that the most effective trap was the lid type placed at a 1 meter, using pheromone plus sugar cane as bait.

Key Words: olfactometric method, trap system, host odor

RESUMEN

En este trabajo se presenta una prueba de campo sobre la actividad de la feromona del gorgojo rayado, *Metamasius hemipterus* (L.), combinada con olores de sus plantas huéspedes y se describe um método olfatométrico que nos permite determinar en el laboratorio la eficacia de los semioquímicos a evaluar y un sistema de trampeo que nos permite determinar en el campo la efectividad de los cebos atrayentes. En el laboratorio se evaluó la respuesta del insecto usando un olfatómetro de caída de dos pocillos. Como fuentes aromáticas se usaron olores de las plantas huéspedes: a) seudotallo y cormo de *Musa* sp. variedad cambur manzano (AAB) y b) tallos de caña de azúcar *Saccharum* sp. Los resultados indican que las aromas de las plantas huéspedes atraen al insecto, que el aroma de la caña de azúcar es más atrayente que el del cormo de cambur manzano, y que el aroma del seudotallo es tan atrayente como el aroma del tallo de la caña de azúcar. Para el estudio de campo se diseñaron dos trampas de captura: trampa con tapa y trampa con embudo. Se evaluó la eficiencia de captura de estas trampas colocadas a dos alturas (0 y 1 metro) y usando como cebo atrayente la feromona de agregación sola, la feromona con el seudotallo de cambur manzano, la feromona con la caña de azúcar, la feromona con el cormo de cambur manzano, y la feromona con piña. Los resultados indican que la trampa más atrayente fue la trampa con tapa colocada a un metro de altura con feromona mas caña de azúcar como cebo.

Due to the problems posed by the use of synthetic insecticides for pest control, including environmental degradation and the pests' development of resistance to insecticides (Getz and Gutierres, 1982; Jaffé et al. 1993), there is a growing use of natural enemies and semiochemicals in integrated insect pest control programs. That approach is of considerable practical utility for controlling some Coleoptera pests (Vilela and Castro, 1987).

The sugar cane weevil *Metamasius hemipterus* (L.) (Coleoptera: Curculionidae) is a serious sugar cane pest (Restrepo et al. 1982). Some authors (Cárdenas, 1976; Boscan and Godoy, 1988) also view it as a secondary agricultural pest for Musaseae. In its larval stage *M. hemipterus* damages sugar cane stalks and Musaseae pseudostems. As it feeds in those zones, it digs tunnels, destroys tissue, and weakens the plant.

The use of poisoned sugar cane and Musascea traps to control these beetles (Restrepo et al. 1982; Nava and Sosa, 1985) has revealed that this species, like many other insects (Bell, 1991), uses volatile semiochemicals probably originating in fermented pieces of Musasea pseudostem and sugar cane stalk to orient it to the plant. Using pitfall traps in southern Florida (USA), Giblin-Davis et al. (1994) showed that the sugar cane weevil is attracted by sugar cane, musasea pseudostem, palm crown, and sugar cane stem aromas.

Pure attractants isolated from host plants or pheromonal compounds may be useful as bait to lure the insects into traps for monitoring or control purposes.

Rochat et al. (1993) used a collection method based on supelpack and desorption with dichloromethane, then gas chromatography coupled with mass spectrometry, to isolate and identify 4 methyl-5-nonanol as the main component of an aggregation pheromone produced by male *M.hemipterus*. The same compound is also present in the aggregation pheromone of another rhynchophorine, *Rhynchophorus vulneratus*. The sugar cane weevil pheromone was subsequently synthesized by Mori et al. (1993).

Traps with attractant semiochemicals, pheromones, and kairomones have been successfully used for coleoptera control of the boll weevil *Anthonomus grandis* Hardee, (1982), wood-attacking scolitids (Borden, 1993), and the coconut weevil (Hernández et al. 1992; Oehlschlager et al. 1993). Hence, massive trapping of sugar cane weevils may be a viable method for control.

Castrillo et al. (1995) reported that the pheromone emitted by the male is (4S,5S) 4-methyl-5-nonanol; (+/-) 2-methyl-4-heptanol) in 8: 1 proportion. In April 1995 we received 50 devices for releasing the pheromone. Each lure will release a constant amount of pheromone per day in the field during 2-3 months (Oehlschlager, pers. comm).

In this study we field tested the behavioral activity of the *M. hemipterus* pheromone mixed with the odors of its host plants, and developed (1) an olfactometric method for laboratory determination of the effectiveness of the semiochemicals and (2) a field trap system with which to determine the effectiveness of baits in attracting sugar cane weevils in the field. Using those olfactometric methods we measured the attraction of sugar cane weevils to semiochemicals from their host plants and the aggregation pheromone described for this species.

Materials And Methods

Capture and Maintenance of Adults.

The insects were collected in a plantation of edible musaseae plants shaded by cacao trees near the Simón Rodríguez University Río Negro Experiment Station in Acevedo District of Miranda State, Venezuela (10°20' North, 66°11 West, at 42 meters above sea level, with 2050 mm of annual precipitation). The plantation produces a mix of banana and plantain varieties including: "pineo" (genotype AAA), "plátano" (AAB), "topocho" (ABB), and "cambur manzano" (AAB).

The place is not a commercial musaseae or sugar cane plantation. Moreover, chemical insecticides or herbicides are not used in the experimental plot.

The insects were caught with sandwich-type retention traps (Castrillón, 1989) which use pseudostem tissues as attractors. The insects were then taken to the laboratory in individual plastic containers 7.5 cm high by 9 cm in diameter, the lid perforated to allow air flow. In the laboratory the insects were kept in plastic containers, in groups of 10. The adults were given a piece of sugar cane for food. The specimens were kept in the laboratory, at 23-27°C and 70-90% relative humidity, with 12 hours of light and 12 of darkness per day. Under these conditions the adults fed twice a week on sugar cane pieces of 80 to 100 g, cut lengthwise.

Olfactometric Methods

The Pavis and Minost (1992) double-pitfall olfactometer (Fig. 1) was adapted to determine attraction of the aggregation pheromone to adults. This device was built as a cylindrical chamber (25 cm in diameter by 30 cm high), connected to two jars containing the odor sources. The duration of each piece of experiment was of six hours (9:00 to 14:00), using 20 insects placed on the platform at a time. The number of insects that

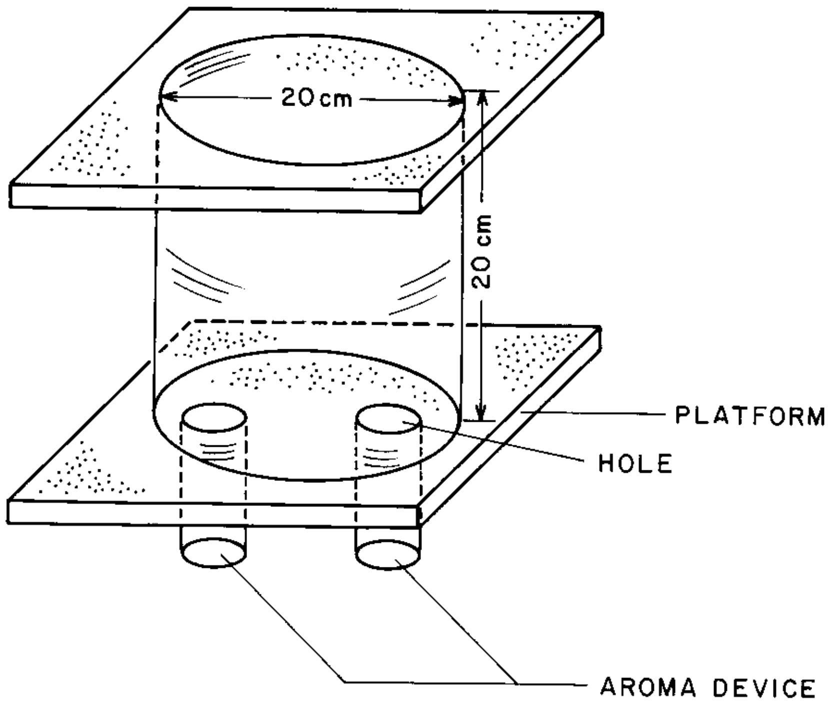

Fig. 1. Double-Pitfall Olfactometer.

had fallen into each jar was counted at the end of each period without distinction by sex. The tests were carried out in a room with dimmed daylight and a normal photoperiod. The environment was kept clean with extractor fans to remove odors not related to the experiment.

Odor Sources

The odor sources used for the biotests were 50 grams of vegetable tissue taken from "cambur manzano" banana clone pseudostems and rhizomes without flowers or fruit (AAB genomic composition), 50 grams of pineapple, and 50 grams of sugar cane stalk (*Saccharum* sp). The vegetable material was collected at the aforementioned Musaseae plantation, located in the vicinity of the Simón Rodríguez University Río Negro Experiment Station.

Field Capture Tests

The traps were placed in a shaded area of an edible Musaseae plantation between May and July 1995, arranged in a straight line perpendicular to the prevailing wind direction, with alternation of the treatments under examination; they were placed approximately at 10 m apart, and checked at 2 or 3 day intervals. The experiments were conducted between May 22 and July 19, 1995.

Two distinct trap designs were tested to compare their capture efficiency:

Design 1: Funnel trap (Fig. 2). This kind of trap is composed of a plastic cylinder 10 cm in diameter by 14 cm high, with a 5 cm-high funnel in its top half. A layer of water approximately 3 cm deep was placed at the bottom of the trap to keep the insects from escaping. The aroma sources were suspended from a wire half way down the cylinder.

Design 2: Perforated lid trap. This trap uses the same plastic cylinder, with no funnel but with a lid having 20 holes approximately 4 mm in diameter. A layer of water approximately 3 cm deep was placed at the bottom of the trap to prevent escape. The aroma sources were suspended from a wire half way down the cylinder.

Evaluation of pheromone device activity.

The following baits were used to evaluate the pheromone's field effectiveness in the pheromone release device (PRD) described by Castrillo et al. (1995) and Camm Oehlsclager pers. comm. in perforated lid trap. Lures of pheromones (4S, 5S) 4-methyl-5-nonanol; (+/-) 2-methyl-4-heptanol) in 8: 1 proportion.

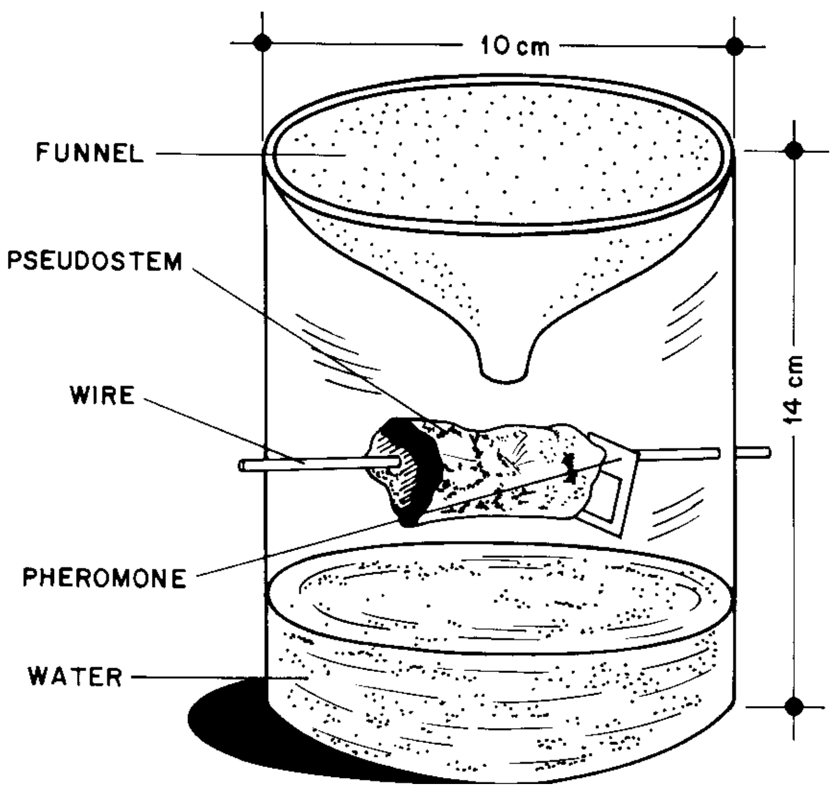

Fig. 2. Funnel Trap.

1- PRD plus 2 to 3 pieces of "cambur manzano" banana pseudostem (approximately 50 g).

2- 2 to 3 pieces of "cambur manzano" banana pseudostem (approximately 50 g).

The traps were arrayed in a straight line at 10 meter intervals, in the shade, with alternating baits. Four traps were used for each treatment and, checked every 2 to 3 days over a 15-day period.

Evaluation of the synergetic effect of the pheromone release device (PRD) combined with aromas from vegetable tissues in perforated lid trap.

A) The PRD plus 2 to 3 pieces of sugar cane stalk (*Saccharum* sp.) without flower or fruit (approximately 50 g). B) The PRD device plus 2 to 3 pieces of pineapple fruit (*Ananas* sp.) (approximately 50 g).C) The PRD plus 2 to 3 pieces of "cambur manzano" banana pseudostem (approximately 50 g). D) The PRD plus 2 to 3 pieces of "cambur manzano" banana rhizome (approximately 50 g). And E) The PRD alone.

The traps were arrayed in a straight line at 10 meter intervals, in the shade, with alternating heights. Four traps were used for each treatment and the traps were checked every 2 to 3 days over a 33-day period.

Evaluation of the two trap designs and the effect of Trap Height.

To evaluate the effect of the traps' height and the trap design on capture efficiency, eight funnel and lid traps were placed at ground level and at a 1 meter height (after Oehlsclager, pers comm). The number of insects trapped was counted every 2-3 day for 44 days. The traps were arrayed at 10 meter intervals, in a straight line, and in the shade, with alternating heights. PRD plus 2 to 3 pieces of pseudostem were used as the bait.

Statistical Analysis

The null hypothesis that there are no differences among the various treatments was tested by the Kruskal Wallis Siegel non-parametric Variance Analysis test (Siegel, 1956). The numerical values or data are the numbers of individuals caught per 2-3 day period for each trap. The Statistix Release 4.0 (Sx 4.0) statistical package, installed in a personal computer, was used for the analysis.

Results And Discussion

Olfactometric Results

Table 1 illustrates the responses of *M. hemipterus* weevil to stimulus by rhizome aroma vs. air, pseudostem aroma vs. air, and sugar cane stalk aroma vs. air. It is clearly shown that the insects strongly prefer the plant odors (rhizome, pseudostem, and sugar cane) to pure air. This indicates that olfactometry permits the determination of whether a particular aroma attracts sugar cane weevils or not, and is the first olfactometric evidence for this species obtained in the laboratory. Several studies have demonstrated the attractiveness of pseudostem and sugar cane stalk odors in the field (Boscan and Godoy, 1982; Boscan and Godoy, 1988; Restrepo et al. 1982 and Giblin-Davis et al. 1994).

Table 1 shows that, under constant humidity, temperature, and dimmed natural light, and having the aromatic volatiles produced by the tissues used in the study as their only source of stimulation, sugar cane weevils are attracted by these aromas, with sugar cane being more effective than rhizome and as effective as pseudostem.

TABLE 1. RESPONSE OF *M. HEMIPTERUS* ADULTS WITHOUT DISTINCTION BY SEX, STIMULATED BY VOLATILES FROM "APPLE" BANANA RHIZOME AND PSEUDOSTEM AND SUGAR CANE STALK.

Comparison of Aroma Source A / Source B	Source A	Source B	No Choice	Total
Rhizome/air	12*	1	7	20
Pseudostem/air	13*	4	6	20
Sugar cane/air	10*	2	8	20
Pseudostem/rhizome	11	6	3	20
Sugar cane/rhizome	16***	1	3	20
Sugar cane/pseudostem	10	8	2	20

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$ for a binomial test between two aromatic sources.

Traps in the Field

Twelve insects were observed to be attracted by the aromatic sources in the field. *M. hemipterus* adults fly at a height of 1 to 2 meters, in a zig-zag course for short distances of 4 to 5 meters before falling near the source of odor, or on it. Then a beetle actively walks into the trap or conceals itself in the brush.

- Types of baits:

Table 2 shows the captures of adult *M. hemipterus* specimens with traps using the PRD plus 2 to 3 pieces of "cambur manzano" banana pseudostem, as well as traps holding only pseudostem. It is clear that the pheromone increased captures.

Table 3 shows the captures of adult *M. hemipterus* with traps using the pheromone release device plus sugar cane stalk PH+CANE, pineapple fruit PH+PINE, "cambur manzano" banana pseudostem PH+PSEUD, "cambur manzano" banana rhizome PH+RHIZ, and pheromone alone PH ALONE. Three groups emerged: 1) pheromone plus sugar cane or pineapple, the one which proved to be the most effective; 2) pheromone plus pseudostem or rhizome; and 3) pheromone alone.

These results suggest that the pheromone requires the synergy of plant odors to be activated and produce the aggregation of *M. hemipterus*. The fact that the aggregation pheromone requires vegetable odor synergy to be active in field conditions appears to

TABLE 2. FIELD CATCHES OF ADULT WEEVILS USING TRAPS WITH PSEUDOSTEM AND TRAPS WITH PSEUDOSTEM PLUS PHEROMONE.

Trap	Captured insects		
	mean per trap ± sd		N
With pseudostem	0.00	0.00	20
With pseudostem plus pheromone	0.18	0.39	39

*$p < 0.05$ for a Kruskal Wallis non-parametric variance analysis applied to the ranges of insects caught, for each treatment.

N = number of catches per 2-3 day period for each trap.

TABLE 3. FIELD CATCHES OF ADULT WEEVILS USING TRAPS BAITED WITH PHEROMONE PLUS PSEUDOSTEM (PH + PSEUD), PHEROMONE PLUS RHIZOME (PH + RHIZ), PHEROMONE PLUS SUGAR CANE (PH + CANE), PHEROMONE PLUS PINEAPPLE (PH+ + PINE), AND PHEROMONE ALONE (PH ALONE). 40 CATCHES PER 2-3 DAY PERIOD FOR EACH TRAP ARE GIVEN.

Trap	Captured insects mean per trap ± sd	Homogeneous Groups*		
PH+CANE	1.15 ± 1.70	I		
PH+PINE	1.12 ± 1.18	I		
PH+PSEUD	0.47 ± 0.85		I	
PH+RHIZ	0.40 ± 0.84		I	
PH ALONE	0.10 ± 0.50			I

*Homogeneous groups for a Kruskal Wallis non-parametric variance analysis applied to the ranges of insects caught for each treatment.

be a common condition among the *Rhynchophorine* species (Cerda et al. 1997, Jaffé et al. 1993), which share many pheromonal components (Rochat et al. 1993).

In Table 4 we shows the number of individuals attracted to the traps of each design type, placed at a 1 meter height and at ground level. The trap which caught the most insects was the lid trap placed 1 meter above the ground.

In Table 4 we observed that the trap with the lid is clearly more efficient, both at 1 meter height and on the ground ($p = 0.000$ for a Kruskal Wallis test applied to the ranges of the insect for each treatment). That may reflect the insect's flight path. Because they approach the trap, halt in mid-air, and fall into the trap, landing on the lid may be easier for the insects than landing on the -funnel.

Table 4 shows that there is a significant difference in the number of beetles caught in traps placed at different heights. The traps with lid at 1 meter above the ground clearly caught more insects than those on the ground ($p < 0.05$ for a Kruskal Wallis test applied to the ranges of the insect for each treatment). Under the conditions of the study, the insect flies at 1 to 2 meters above the ground, and landing may be easier on the elevated traps than on ground-level traps.

TABLE 4. FIELD CATCHES OF ADULT WEEVILS USING FUNNEL TRAPS AND LID TRAPS PLACED AT A 1 METER HEIGHT AND AT GROUND LEVEL. 68 CATCHES PER 2-3 DAY PERIOD FOR EACH TRAP ARE GIVEN.

Trap	Captured insects mean per trap ± sd
Funnel trap at 1 meter height	0.03 ± 0.17
Funnel trap at ground level	0.00 ± 0.00
Lid trap at 1 meter height	0.21 ± 0.40
Lid trap at ground level	0.06 ± 0.24

We conclude that, under the conditions of the study, the sugar cane weevil is attracted by host plant aromes with sugar cane being more effective than musaceae rhizome and as effective as musaceae pseudostem, and that RPD requires the synergy of plant odors to be activated and produce the aggregation of *M. hemipterus*. The lid traps at 1 meter above the ground clearly caught more insects than those on the ground. This may be related with the weevil's flight behaviour.

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