Diaprepes abbreviatus[1]: Laboratory and Field Behavioral and Attractancy Studies[2]

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ABSTRACT

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In laboratory olfactometer tests conducted in a dark room, male and female *Diaprepes abbreviatus* (L.) (Curculionidae: Coleoptera) were shown to be significantly attracted to the frass of the opposite sex, though some response to the same sex was obtained. Response was also obtained from males and females to volatiles of tender, succulent citrus foliage. Electroantennagram studies of extracts of frass and foliage indicated that the adult weevils were stimulated by these materials. In field tests, traps baited with a combination of males and females were significantly more attractive than were traps baited with males or females only, or unbaited traps.

Diaprepes abbreviatus (L.), commonly called the West Indian sugarcane rootstalk borer, is a major pest of citrus and sugarcane in Puerto Rico and the West Indies. It was first discovered attacking citrus in Orange County, Fla., in 1964 (Woodruff 1964). In 1968, a quarantine area was established which presently includes ca. 20,000 ha in Orange and Seminole Counties, as well as a small area near Ft. Lauderdale, Broward County, Fla. The adult weevils feed on the young, tender foliage of citrus and other host plants and deposit their eggs in masses between mature leaves, which are held together by an adhesive secretion. The neonate larvae drop to the soil and burrow in, where they remain for 1 to 2 years and cause serious root injury to the host plant.

Field observations in Florida indicate that adult *D. abbreviatus* congregate on or near new growth on the citrus trees. Trees without new or tender foliage usually have few or no weevils present. In Puerto Rico, Wolcott (1936) reported that host trees such as the Moca, *Andira inermis* (Wright) HBK, which produce all their new leaves at the same time, are often completely defoliated by *D. abbreviatus*. Also, adult weevils often prefer certain trees to others of the same kind nearby, and the weevils will remain congregated on such trees for weeks at a time. Beavers and Selhime (1978) released marked weevils in an isolated grove in Florida and observed that the same weevils remained on the same trees for up to 2 weeks.

Results of tests with several sizes and colors of wing-type traps baited with males or females have been inconclusive in determining the presence of an attractant in this weevil (Beavers, unpublished data).

The purpose of these studies conducted from 1975 to 1978 was to determine if an attractant was present in *D. abbreviatus* and to develop laboratory and field bioassay techniques for this attractant.

Materials and Methods

Adult weevils used for the bioassay tests were collected from the field, sexed, and held separately in screen cages (25 by 30 by 25 cm). They were fed citrus foliage. The cages were held in a screenhouse (6 by 6 by 2 m) covered with a corrugated fiber glass roof. Frass was collected daily from the bottom of the cages and from the citrus foliage, weighed, and stored initially in a variety of solvents. Later, the collected frass was stored in hexane. The fecal material was put through three extraction procedures. (1) The storing hexane was decanted, and the residue was washed three times with small quantities of fresh hexane. The washes were combined, passed through a 2.5-cm plug of anhydrous sodium sulfate, and then diluted to 10 or 100 ml, depending on the quantity of fecal material that was available. (2) The fecal residue was then shaken in a ball mill with hexane for ca. 10 min. The solvent was filtered, and the process was repeated two times. The extracts were combined and treated as in procedure 1. (3) Procedure 2 was repeated, using reagent-grade acetone as a solvent.

The crude extracts were concentrated to a volume of ca. 0.5 to 1 ml and fractionated through 10 g of silica gel (60- to 200-mesh) held in a 250-mm-length column (12 mm I.D.). Hexane, 2, 5, 15, and 50% ether-hexane, ether, and methanol (30 ml of each) were used sequentially as eluants. Fractions (10 ml each) were collected and concentrated before testing.

New, succulent citrus foliage was collected, weighed, and stored in six separate solvents: hexane, heptane, xylene, methylene chloride, ethanol, and 1,4-dioxane. Concentrated extracts were fractionated by the same procedure used for the frass.

Porapak Q was conditioned in a manner similar to that described by Byrne et al. (1975). The Porapak Q collections were made by drawing air over citrus foliage contained in a 2.8-liter glass jar. A Porapak Q column prefiltered the airstream, and the volatiles were collected on a second column as the airflow

[1] *Diaprepes abbreviatus* (L.) Coleoptera: Curculionidae
[2] Received for publication 6 April 1981. This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by the USDA, nor does it imply registration under FIFRA as amended. Also, mention of a commercial or proprietary product does not constitute an endorsement by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
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left the jar. Absorbed volatiles were
extraction.

Laboratory Tests

Olfactometer Tests.—The olfactome
by Hardee et al. (1967) for the boll we
omus grandis Boheman, was modifie
5.0-cm-diameter hole in the glass pla
as the central arena. This enabled easie
and removal of the weevils and clear
factometer after each test. An identi
without the hole was clamped onto
the olfactometer and served as the
weevils. Ten insects were placed in
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contained either the candidate attra
trol. Tests were run for 1 h in a dark r
response consisted of the weevils m
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placing 50 weevils of one sex into
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foliage tests. In the adult tests, fi
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Because the odor of citrus folia
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individual component volatiles kn
in citrus foliage (Kesterson et al.
These included α-Pinene, camphe
cene, ε-3-carene, α-terpinene,
nene, p-cymene, methyl hepte
decanal, linalool, menthone, β-ca
pineol, neryl acetate, nerol, ger
ocimene.

EAG Tests

The methods for recording t
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(1971) and Adler and Jacobson
modification. The recording ele
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It became apparent that the
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of the day. It was found that res
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Field Tests.—A cylindrical h
mesh) trap, similar to the modi
trap (Beavers et al. 1979), w
and 22.5 cm in diameter wi
(37.5 by 37.5 by 0.6 cm) at
bottom and coated with Tac

the jar. Absorbed volatiles were removed by extraction.

Laboratory Tests

Olfactometer Tests.—The olfactometer described by Hardee et al. (1967) for the boll weevil, *Anthonomus grandis* Boheman, was modified by cutting a 3.0-cm-diameter hole in the glass plate that served as the central arena. This enabled easier introduction and removal of the weevils and cleaning of the olfactometer after each test. An identical glass plate without the hole was clamped onto the bottom of the olfactometer and served as the arena for the weevils. Ten insects were placed in each olfactometer. The bioassay of a candidate compound was replicated with a series of 10 olfactometers. Air was pulled into the arena from two tubes opposite each other. The tubes were connected to flasks which contained either the candidate attractant or a control. Tests were run for 1 h in a dark room. A positive response consisted of the weevils moving from the arena and into a flask containing the candidate attractant. Frass-contaminated foliage was obtained by placing 50 weevils of one sex into a 2.8-liter paper carton with a citrus foliage bouquet overnight. Then 5 g of the foliage was used as the bait in each olfactometer. A bouquet without weevils was held and weighed in the same manner for the uncontaminated foliage tests. In the adult tests, five weevils of the opposite sex were used as the adult attractant source.

Because the odor of citrus foliage elicited a positive response in the olfactometer, several of the individual component volatiles known to be present in citrus foliage (Kesterson et al. 1971) were tested. These included α-Pinene, camphene, β-pinene, myrcene, ε-3-carene, α-terpinene, γ-terpinene, limonene, p-cymene, methyl heptenone, citronellal, decanal, linalool, menthone, β-caryophyllene, α-terpineol, neryl acetate, nerol, geraniol, thymol, and ocimene.

EAG Tests

The methods for recording the electroantennagram (EAG) were those described previously by Adler (1971) and Adler and Jacobson (1971), with a slight modification. The recording electrode either penetrated the antennae through a small puncture at the tip of the antenna, or a larger electrode capped the antennal tip. Both methods produced equally satisfactory results.

It became apparent that the electrical responses obtained during morning hours were small, and it was decided to record the responses at various times of the day. It was found that responses obtained were larger in magnitude during the evening hours than those obtained during the morning hours. Thereafter, all testing was done during the evening hours.

Field Tests.—A cylindrical hardware cloth (0.3-cm mesh) trap, similar to the modified Rid-O-Ray® light trap (Beavers et al. 1979), which was 37.5 cm high and 22.5 cm in diameter with a piece of plywood (37.5 by 37.5 by 0.6 cm) attached to the top and bottom and coated with Tack Trap®, was used in the field tests. The traps were suspended at a height of 2 m and spaced 7.5 m apart in a single E-W row adjacent to a plot of weevil-infested juniper, Juniperus communis L. The traps were set in randomized complete block replicated three times. The males and females were held in separate cages for 30 days before the tests were initiated. A perforated, 1-liter carton was suspended inside each trap and contained either 25 females, 25 males, 25 females and 25 males, or an unbaited trap. Citrus foliage was placed in each carton, and fresh foliage was provided weekly when captured weevils were sexed, counted, and recorded from 7 August to 5 September 1978.

In a second field test conducted during August and September 1980, a period of low weevil population, seven compounds which had previously attracted one or two weevils during field testing in Puerto Rico (unpublished data), and which also had elicited distinct EAG responses, were tested. These compounds were: Z-3-hexen-1-ol, 1-methyl-1,2-ethanediyl propionate, 1-methyl-4-(2-methyl-2-oxiranyl)-7-oxabicyclo[4.1.0]heptane, linalool oxide, 5-methyl-2-(1-methylethenyl)cyclohexanol, 2-(2-octenyl)cyclopentanone, and isoquinoline.

Results

Laboratory Olfactometer Tests

The olfactometers baited with adult weevils as the attractant source elicited no response when weevils of either the opposite or the same sex were placed in the arena. Feral- and laboratory-reared, virgin weevils were exposed as the attractant to feral and virgin weevils in separate tests. However, when citrus foliage containing residues of frass from females was used as the attractant source, a mean index of attraction (IA) (Tumlinson et al. 1968) of 0.40 for males and 0.22 for females was obtained. When citrus foliage containing residues of frass from males was the attractant source, an IA of 0.30 for males

Table 1.—Response of adult *D. abbreviatus* in laboratory olfactometers to frass of the opposite sex and to citrus foliage[a]

Attractant	Sex released[b]	
	Females	Males
Female frass		
Insects	112	132
Check	73	52
No response	65	66
IA	0.22	0.40
Male frass		
Insects	150	120
Check	62	62
No response	38	68
IA	0.47	0.30
Citrus foliage		
Insects	149	132
Check	72	79
No response	29	39
IA	0.43	0.31

[a] One-hour test in dark room (25 replicates, 10 insects per replicate).
[b] Index of attraction (Tumlinson et al. 1968).

and 0.47 for females was obtained. When uncontaminated foliage was used as the attractant, an IA of 0.31 for males and 0.43 for females was obtained. When foliage contaminated by frass from either the same or the opposite sex was tested vs. uncontaminated foliage, the response was about equal and no IA was obtained. Although the weevils had a significantly higher IA for foliage contaminated by frass from the opposite sex ($P = 0.05$, t test), the IA for uncontaminated foliage was not significantly different for either sex.

When 0.1 g of dry fecal material collected over a period of several days was exposed to the same or the opposite sex in the olfactometers, no response was elicited.

Although crude methylene chloride extracts of fecal material, as well as crude extracts of citrus foliage from various solvents, indicated attraction to adults in the olfactometer, fractionation of the extracts by column chromatography resulted in variable test results. Crude extracts of citrus foliage with 1,4-dioxane elicited the greatest response among the various solvents used (IA = 0.22). However, a large portion of the attractive material(s) in the 1,4-dioxane extract could be partitioned into hexane. The IA of the extractive hexane layer was 0.27, whereas the IA of the hexane-extracted 1,4-dioxane was 0.24.

Collections of volatiles from adults, frass, and citrus foliage on Porapak Q were also made. A hexane extract of the Porapak Q citrus leaf volatiles resulted in an IA of 0.29 and 0.32 when adults were exposed to rates of 10 and 50 µl, respectively.

None of the component volatiles of citrus foliage tested alone proved to be attractive. We also tested over 100 other candidate terpene compounds without results.

EAG Tests

Because the adult weevils were available for only a portion of the year in the large numbers required for adequate replications in the olfactometer, and only small amounts of materials were available for testing, EAG tests were initiated. Results of these tests confirmed previous findings with the olfactometers. Distinct EAG responses were obtained from each sex to extracts of fecal material and to citrus leaves. In tests conducted at 0900, 1500, and 2100 h, the male response to both the hexane and acetone extracts of fecal material was distinct and constant. The female also responded to both of these extracts; however, the response to the hexane extract continued to increase in intensity from the 0900 to the 2100 h tests. In tests made at 0900 h EST, the male response was greater than the female, at 1500 h the female response exceeded that of the males, and at 2100 h the female response was two to three times greater than the male, indicating increased nocturnal activity of the females. In tests with fractionated fecal extracts, both sexes showed a consistent response of moderate intensity to materials eluted with the polar solvents (50 and 100% ether). Low EAG responses were obtained when live adults or adult washes were exposed to the same or the opposite sex.

Field Tests

Traps baited with males or females caught a mean of 23.6 ± 3.8 and 9.9 ± 4.2 weevils per trap, respectively. Traps baited with a combination of males and females or controls caught a mean of 32.0 ± 4.0 and 13.3 ± 7.6 weevils per trap, respectively (Fig. 1).

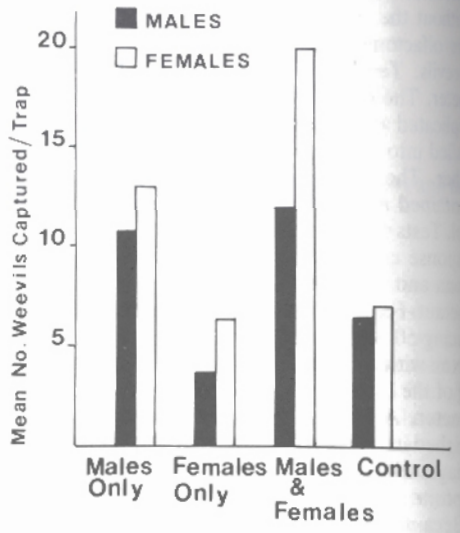

FIG. 1.—Field response of *D. abbreviatus* adults to traps baited with males only, females only, males and females, and control; 7 August to 5 September 1978, Plymouth, Fla.

Analysis of variance showed there was a significant difference in the attractiveness ($P = 0.05$, Duncan's multiple range test) between traps containing both males and females, and males only, females only, and the controls.

The number of females was higher than the number of males caught per trap for each attractant source. The traps baited with a combination of males and females caught about twice as many females as males (Fig. 1), indicating greater activity by the females, although it could not be statistically demonstrated in this test or in previous olfactometer tests where female response was also found to be greater.

One of the seven field-tested compounds (isoquinoline) attracted 2 females, whereas the standard trap baited with 25 males also caught 2 females over a 3-day period. No weevils were caught on the unbaited trap.

Discussion

Significant attraction to the residues of the opposite sex on citrus foliage was exhibited by both male and female *D. abbreviatus* in olfactometer tests. Each sex was also attracted to volatiles from uncontaminated citrus foliage. These responses were confirmed in EAG tests of extracts of frass and citrus foliage. Odors from adult weevils did not elicit a

significant response in either the olfactometer tests. Results of these tests indicate the p an attractant in the weevil frass which a opposite sex as well as the same sex, but degree. A situation similar to this was r Godbee and Franklin (1976), where the of an aggregation pheromone was demon virgin females and males of the black turp tle, *Dendroctonus terebrans* (Olivier). A gism between pheromones and host pla has been reported for several other spec 1971, Werner 1972, Billings et al. 1976 et al. 1977, Flint et al. 1979).Mitchell demonstrated that the attractiveness of t vil was closely related to the passage of f and that the frass was more attractive th

Young citrus foliage was equally attra sexes, suggesting that it acts as a host-f the aggregation of this weevil. This served with other plant hosts for *D. abb* they are found on the plants only d when new growth is present. Del Foss (1977) in laboratory olfactometer studi the water hyacinth weevils, *Neochet* Warner and *N. bruchi* Hustach, and mites, *Orthogalumna terebrantis* Wall nificantly attracted to young, growing hyacinth.

In our tests, individual componer liage did not elicit response by *D. ab* ever, in subsequent tests (unpublis adults of either sex were individually extracts of citrus foliage dispensed blocks, 80% of the females and 70 were able to locate and attempt to block in a 1-m³ cage held in a dark

Therefore, it is suggested that *D.* initially locate new growth of cit other host plants by olfactory per subsequent feeding and passage apparently releases the volatile at opposite sex. This would explai behavior of *D. abbreviatus* on a other nearby hosts are not attack

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Adler, V. E. 1971. Physical conditi reproducibility of electroantenn mol. Soc. Am. 63: 300–302.

nificant response in either the olfactometer or EAG ts. Results of these tests indicate the presence of attractant in the weevil frass which attracts the posite sex as well as the same sex, but to a lesser gree. A situation similar to this was reported by odbee and Franklin (1976), where the production an aggregation pheromone was demonstrated for gin females and males of the black turpentine bee- *Dendroctonus terebrans* (Olivier). Also, syner- m between pheromones and host plant terpenes as been reported for several other species (Pitman 971, Werner 1972, Billings et al. 1976, McKibben et al. 1977, Flint et al. 1979).Mitchell et al. (1975) monstrated that the

the aggregation of this weevil. This has been observed with other plant hosts for *D. abbreviatus*, i.e., they are found on the plants only during periods when new growth is present. Del Fosse and Perkins (1977) in laboratory olfactometer studies showed that the water hyacinth weevils, *Neochetina eichorniae* Warner and *N. bruchi* Hustach, and water hyacinth mites, *Orthogalumna terebrantis* Wallwork, were significantly attracted to young, growing tissue of water hyacinth.

In our tests, individual components of citrus foliage did not elicit response by *D. abbreviatus*. However, in subsequent tests (unpublished data) when adults of either sex were individually exposed to crude extracts of citrus foliage dispensed on balsa wood blocks, 80% of the females and 70% of the males were able to locate and attempt to feed on a single block in a 1-m^3 cage held in a dark room.

Therefore, it is suggested that *D. abbreviatus* may initially locate new growth of citrus foliage or of other host plants by olfactory perception; then the subsequent feeding and passage of fecal materials apparently releases the volatile attractant(s) for the opposite sex. This would explain the aggregation behavior of *D. abbreviatus* on a specific host while other nearby hosts are not attacked.

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