OVIPOSITIONAL PREFERENCES OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Female *Diaprepes abbreviatus* L. were presented with immature citrus leaves, mature citrus leaves, and doubled strips of various substances as potential oviposition sites. In both the laboratory and in caged outdoor experiments more egg masses were deposited between freezer paper strips than between mature leaves. No egg masses were deposited between immature leaves. Artificial substrates were preferred in the following order: freezer paper > waxed paper > transparency film. In emergence experiments utilizing freezer paper with the plastic-coated side out as the oviposition substrate, no neonate larvae escaped from 65% of the egg masses. In contrast, larvae easily escaped from egg masses laid between strips of the other substrates tested. In the field, wax paper and freezer paper were preferred as oviposition sites 43:1 and 50:1 over mature leaf pairs, respectively. These data suggest a new prototype monitoring trap for this weevil.

Key Words: oviposition trap, insect-plant interaction, root weevil, management practice

RESUMEN

Se presentaron a hembras de *Diaprepes abbreviatus* L. hojas jóvenes y maduras de cítrico y franjas dobles de varios tipos de papel como sitios potenciales para oviposición. Tanto en experimentos en el laboratorio como dentro de jaulas en el campo, más huevos fueron depositados entre las tiras de papel para congelar que entre las hojas maduras. Ninguna masa de huevos fué depositada entre las hojas jóvenes. Los substratos artificiales fueron preferidos en el orden siguiente: papel para congelar > papel encerado > papel de transparencias. En experimentos de surgimiento utilizando papel para congelar (con la cubierta plástica hacia el exterior), ninguna larva neonatal se escapó del 65% de las masas de huevos. Por el contrario, las larvas se escaparon fácilmente de las masas de huevos depositadas entre las tiras de los otros substratos. En experimentos hechos en el campo, el papel encerado y el papel para congelar fueron preferidos sobre las hojas maduras como sitios de oviposición a razón de 43:1 y de 50:1, respectivamente. Estos datos sugieren un nuevo prototipo de trampa para el monitoreo de este picudo.

Diaprepes abbreviatus L. is a pest of citrus, sugarcane, and other economic crops of subtropical and tropical areas of the United States and several Caribbean island nations. Adults feed on young foliage, females lay egg clusters between leaves, and the larvae feed on the roots (Fennah 1942, Woodruff 1968). Host plants associated with all life stages of this pest include about 270 species in 157 genera in 59 plant families. These associations range from adult and larval feeding to presence of egg masses. Life cycle hosts (egg to adult development) include *Citrus* spp., peanut; sorghum, guinea corn, corn, Surinam-cherry, dragon tree, sweet potato, and sugarcane. More than 40 plant species in 20 families, are associated with larval feeding (Simpson et al. 1996). Because of inadequate management strategies and a wide range of adult and larval food plants, *D. abbreviatus* can be considered a major long-term threat to the survival of several agronomic crops (Simpson et al. 1996). Tree symptoms do not appear until the larvae are well established on the tree roots.

A single adult *D. abbreviatus* L. was discovered in a citrus nursery in Orange County, Florida in 1964 (Woodruff 1964). Although the nursery was placed under surveillance, no other *D. abbreviatus* was observed until 1968, when larvae were collected from the same nursery (Woodruff 1968). Several hundred adults and larvae were subsequently collected in and around Apopka, Florida (Woodruff 1968). By 1975, this pest was established in 4,300 acres of citrus in Orange, Seminole, and Broward counties (Griffith 1975). Today *D. abbreviatus* infests about 50,000 acres of commercial citrus in 17 Florida counties and 94 commercial and ornamental plant nurseries (Hall 1995).

During their arboreal lifetime of about 4 months (after emergence from the soil), females oviposit approximately 60 egg masses of 30 to 260 eggs each with an average lifetime total of about 5,000 eggs (Wolcott 1936). Fennah (1942) found that *D. abbreviatus* preferred to oviposit between paper strips over tin foil strips and mature leaves, both of which were preferred to young leaves. Wolcott (1933) stated that eggs were overwhelmingly laid between paper strips compared to leaves in the field and that larvae were trapped in the egg mass.

The purpose of this study was to investigate the ovipositional preferences of *D. abbreviatus* for artificial media in the laboratory and in citrus groves.

Materials and Methods

Fourteen experiments of various types were conducted in this study. One experiment tested for congregational preference based on leaf maturity. Several experiments examined ovipositional preference of *D. abbreviatus* for various artificial substrates and for leaf maturity in the laboratory. One type of artificial substrate was compared to mature leaves of young red grapefruit trees in the laboratory, while two experiments repeated others done in the laboratory under semi-controlled and field conditions. A final experiment examined the escape incidence of neonate larvae from egg masses deposited between various types of artificial substrates.

Adult *D. abbreviatus* were field collected at the Kerr Center for Sustainable Agriculture, Vero Beach, Florida, and maintained on a diet of freshly collected red grapefruit leaves (*Citrus* × *paradisi* Macfad. cultivar 'Ruby'). Immature leaves were defined as 75% expanded, but lighter green compared to 100% expanded, dark green, mature leaves. The experiments in Tables 2 and 3 were performed in 30 × 30 × 30 cm aluminum screen cages (BioQuip Inc., Gardena, CA). For the artificial substrates described in Table 1, a 2.5 cm × 30 cm strip was folded in half over a bent paper clip and then stapled once at the open end. Strips were then hung from the top of the cage by the bent paper clip. To provide weevil access to the strip, another unbent paper clip was clipped to the bottom of each strip and inserted into the mouth of an empty 25 ml Erlenmeyer flask. Leaves were paper clipped or stapled together to form pairs and suspended from the cage top in a similar fashion. Substrates being compared to one another were hung in opposite corners of the cage. An empty 25 ml Erlenmeyer flask was placed in a third

TABLE 1. LEAF AND SUBSTRATE DESCRIPTION.

Substrate	Description	Source
Immature leaves	75% expanded	Red grapefruit leaves (*Citrus* × *paradisi* Macfad. 'Ruby')
Mature leaves	100% expanded	Red grapefruit leaves (*Citrus* × *paradisi* Macfad. 'Ruby')
Waxed paper	Tissue paper with a triple coat of paraffin wax	Reynolds Metals Co., Richmond, VA 28261. 800-433-2244
Transparency film	Polyester film (4 mil)	Labelon Inc., Cananadaigua, NY 800-428-5566
Freezer paper	Polyethylene plastic (0.4 mil) on one side of 35 lb wet strength craft paper	Reynolds Metals Co., Richmond, VA 28261. 800-433-2244
Parafilm M®	Sheet of 44% polyolefin/ 56% paraffin wax	Amer. National Can., Greenwich, CT 06836. 203-845-6304
Chromatography paper	Cellulose paper; Whatman® #4	Whatman, Inc., 9 Bridewell Place, Clifton, NJ 07014. 800-441-6555
Vegetable parchment	Parchmentized pure cellulose base paper (2.14 mil)	Sibille Ahlstrom, Inc. 1 Corporate Place, 55 Ferncroft Rd, Danvers, MA 01923. 508-777-9888
Flagging ribbon	Polyvinyl Chloride (6 mil)	United Tape Co., 2545 Ivy Street East Cumming, GA 30131. 800-241-5380

corner of the cage. To provide a food source, 12 cm of terminal growth of immature red grapefruit leaves were placed in the center of the cage. Adult *D. abbreviatus* were placed in the cages at approximately 4:00 pm daily; 16 h later, the number of egg masses between the layers of each substrate type were counted. Each cage was considered one replication. Environmental conditions were 27 ± 2°C, and a 8:16 h light-dark cycle. The experiments in Table 2 and numbers 1 and 2 in Table 3 were conducted at 50% relative humidity (RH). Experiments 3 through 9 (Table 3) were conducted at 27 ± 3°C and a RH of approximately 70% by surrounding cages with containers of water and covering them with 3 mil plastic sheeting. Dependent on weevil availability, 3-

TABLE 2. LEAF MATURITY PREFERENCE FOR CONGREGATION OF ADULT *DIAPREPES ABBREVIATUS* BASED ON THE NUMBER OF ADULTS LOCATED ON GRAPEFRUIT LEAVES AFTER 16 H.

Substrate	n[1]	Total Adults Observed on Target	Mean ± SD Adults/Substrate	Preference Ratio
Immature leaves	63	123	1.95 ± 1.42	17.7
Mature leaves	63	20	0.32 ± 0.69	2.91
Plain flask	63	7	0.11 ± 0.36	1.00

[1]Five males and 5 females per replicate.

TABLE 3. LABORATORY COMPARISON OF EGG MASS DEPOSITION BY *DIAPREPES ABBREVIATUS* BETWEEN VARIOUS SUBSTRATES.

Experiment Number and Substrate		n[1]	Total Egg Masses	Mean ± SD[2] Egg Masses/ Substrate	Preference Ratio
1.	Wax paper	21	11	0.52 ± 0.60 a	5.5
	Mature leaves	21	2	0.10 ± 0.30 b	1
	Immature leaves	21	0	0	0
2.	Freezer paper	50	115	1.15 ± 0.69 a	5.00
	Mature leaves	50	23	0.23 ± 0.32 b	1.00
3.	Freezer paper (on seedlings)	40	50	1.25 ± 1.24 a	12.50
	Mature leaves (on seedlings)	40	26	0.10 ± 0.15 b	1.00
4.	Freezer paper	75	86	1.15 ± 0.97 a	4.79
	Wax paper	75	34	0.45 ± 0.64 b	1.88
	Parafilm M®	75	34	0.45 ± 0.70 b	1.88
	Transparency film	75	18	0.24 ± 0.52 b	1.00
5.	Freezer paper	75	89	1.19 ± 1.14 a	3.79
	Chromatography paper	75	69	0.92 ± 0.78 a	2.98
	Wax paper	75	36	0.48 ± 0.72 b	1.50
	Transparency film	75	24	0.32 ± 0.57 b	1.00
6.	Dry chrom. paper	22	38	0.86 ± 0.54 a	1.56
	Moist chrom. paper	22	24	0.55 ± 0.49 b	1.00
7.	Freezer paper	30	23	0.38 ± 0.43 a	2.11
	Flagging ribbon	30	11	0.18 ± 0.33 a	1.00
8.	Freezer paper	25	34	0.68 ± 0.52 a	1.03
	Parchment paper	25	33	0.66 ± 0.57 a	1.00
9.	6.25 cm freezer paper	60	98	0.82 ± 0.66 a	1.41
	2.5 cm freezer paper	60	69	0.58 ± 0.64 b	1.00

[1]Three males and 3 females per replicate (#1); four males and 4 females per replicate (#4 and #6); one male and 5 females per replicate (#2, 3, 5 and 7-9).

[2]Means ± SD followed by the same letter are not significantly different by Tukey's HSD test at $\alpha = 0.05$. Comparisons were made only within experiments.

5 females were used in each replicate. Generally, only one male weevil was used per replicate. The number of weevils per replicate is noted in the tables.

Attractants (Table 4) were tested by placing either immature or mature grapefruit leaf cuttings or a 16 h collection of frass from 30 *D. abbreviatus* adults (both sexes) which was extracted with hexane (1:10 frass:hexane weight/volume) between individ-

TABLE 4. EGG MASS (EM) DEPOSITION BY *DIAPREPES ABBREVIATUS* ON FREEZER PAPER USING PRESUMPTIVE ATTRACTANTS.

Substrate	n[1]	Total No. of Egg Masses	Mean ± SD Egg Masses/Rep	Preference Ratio
Control[2]	35	30	0.86 ± 1.06	1.16
Immature grapefruit leaves	35	30	0.86 ± 0.88	1.16
Mature grapefruit leaves	35	28	0.80 ± 1.02	1.08
Frass extract	35	26	0.74 ± 0.81	1.00

[1]Five females and 1 male per replicate.
[2]Freezer paper alone.

ual freezer paper strips. One hundred µl of the frass extract was placed on a 5 × 8 cm piece of chromatography paper and inserted between a freezer paper strip. A non-treated 5 × 8 cm piece of chromatography paper was placed between a fourth freezer paper strip as a control (Table 4).

Custom made wooden screen cages (34 × 34 × 108 cm) were placed over five containerized, ≤90 cm tall red grapefruit trees (Table 3, #3). Oviposition sites were artificially prepared by twist-tying leaf pairs together. One 6 cm × 45 cm strip of freezer paper, plastic side out, was folded in half and stapled on the ends and sides and hung in the tree among the leaves. Five females and one male were placed in each cage. No food source was provided to the weevils. Each experiment was conducted from 4:30 pm to 8:30 am (16 h) and was replicated 40 times. Each containerized nursery tree was used in the laboratory for 5 days and then placed outdoors (15 trees were used in the study).

The same cages and trees were set outdoors next to a building on a plywood platform (Table 5). This location experiences approximately 5 h of direct sunlight per day. Each tree had four leaf pairs twist-tied together. One strip of each type of substrate (freezer paper with the plastic coated side out, wax paper, and transparency film) was placed in each tree. Strips were 6.25 cm × 30 cm and were stapled twice at the bottom and twice along the sides. A bent paper clip was stapled to the top of each test material to act as a hanger. All strips were "weathered" by hanging them outside 3-6 days prior to being tested. At 4:30 pm, five females and one male were placed at the base of the

TABLE 5. OUTDOOR CAGE COMPARISON OF OVIPOSITION PREFERENCE OF *DIAPREPES ABBREVIATUS* FOR FOUR SUBSTRATES ON YOUNG CONTAINERIZED AND CAGED GRAPEFRUIT TREES.

Substrate	n[1]	Total No. of Egg Masses[2]	Mean No. Egg Masses/Rep	Preference Ratio
Freezer Paper	30	33	1.10 ± 1.06	18.33
Wax Paper	30	23	0.77 ± 0.97	12.67
Mature Leaves	30	40	0.33 ± 0.26	5.50
Transparency Film	30	2	0.07 ± 0.25	1.00

[1]Five females and 1 male per replicate.
[2]One of each artificial substrate type and 4 leaf pairs per replicate.

trunk and released. The following morning weevils were collected from the cages, and the strips were removed and examined for egg masses. Strips with egg masses were labeled, hung on a wire near the cages, and the number of egg masses and their location was recorded (Table 5). After 3 weeks, each strip with egg masses was examined visually for emergence of neonate larvae.

One each of 6.5×30 cm strips of freezer paper, wax paper, and transparency film was placed in 20 randomly selected 'Minneola' tangelo trees (*Citrus* × *tangelo* J. Ingram & H. E. Moore 'Minneola') (Table 6). Each strip was placed approximately 6-8 inches inside the outside edge of the canopy, halfway down vertically, and equidistant from the other strips in the tree. Ten or more of each type of strip were also placed 50 m distant in a nonexperimental tree so that an extra source of weathered strips would be available as needed. Oviposition strips and all leaves were examined weekly for the presence of egg masses over a period of 6 weeks. Number of egg masses and their location were recorded. All egg masses found on leaves were removed and destroyed. Strips with egg masses were removed from the tree and replaced with an equally weathered strip. The number of leaf pairs suitable for oviposition by *D. abbreviatus* was estimated by the following procedure: five of the 20 trees used in the experiment were randomly chosen, and the number of suitable leaf pairs were counted in one quadrant of each tree. A leaf pair in this case was defined as two leaves which were within 1.5 cm of one another and positioned at an angle other than 90 degrees. This number was then multiplied by 4 (four quadrants), and then divided by 5 to estimate the mean number of potential leaf pairs suitable as oviposition sites per tree.

The trapping effect of artificial substrates was tested for each of the five media types by allowing adult female *D. abbreviatus* to oviposit between wax paper, transparency film, freezer paper with the plastic coated side out, freezer paper with the plastic coated side in, and Parafilm M strips (Table 7). Egg masses were collected daily. Strips containing egg masses were removed from the cage, trimmed with scissors to 2.5 cm on both sides of the egg mass margins, and placed into 4 oz plastic soufflé cups (No. PC400, Fabri-Kal Corp., Kalamazoo, MI 49001) with a moist ½ sheet of KimWipe® (Kimberley-Clark, Roswell, GA 30076) rolled into a loose ball. The collection dates for each egg mass and substrate type were recorded and each was observed daily for egg hatch and neonate larval emergence from the egg mass. One neonate larva per egg mass successfully emerging from the mass was recorded as an escape. Data were compared with the GLM procedure followed by Tukey's HSD test where appropriate (SAS Institute, Inc. Cary, NC).

RESULTS

For congregation, both sexes of weevils preferred young grapefruit leaves over mature leaves by a ratio of 6 to 1 (Table 2). In laboratory oviposition preference experiments, *D. abbreviatus* preferred wax paper over isolated mature leaves 5.5 to 1 and did not oviposit between immature leaves (Table 3, #1). Abreu-Rodriquez & Escolar (1983) found wax paper strips were preferred for oviposition over the foliage of several host plants. Freezer paper was also preferred over isolated mature leaves (5-13 to 1, Table 3, #2 and 3). When freezer paper was compared to the other types of artificial media, it was preferred with the exception of chromatography paper (Table 3, #4 and 5). *D. abbreviatus* exhibited a preference for dry over moist chromatography paper (Table 3, #6), but no preference for freezer paper over flagging ribbon obtained from the field (Table 3, #7). Freezer and parchment paper were chosen equally (Table 3, #8). However, the adhesive deposited by the female during oviposition did not hold the parchment paper together around the egg mass. Weevils preferred 6 cm wide freezer

TABLE 6. FIELD OVIPOSITION PREFERENCE OF *DIAPREPES ABBREVIATUS* ON 'MINNEOLA' TANGELOS.

Substrate	Total Egg Masses	n = 120[1] Mean Egg Masses/Rep.	n = 20[2] Mean Egg Masses/Rep.	n = 14[3] Mean Egg Masses/Rep.	Preference Ratio[4]
Freezer paper	7	0.06 ± 0.30	0.35 ± 0.67	0.50 ± 0.76	50.0
Wax paper	6	0.05 ± 0.25	0.30 ± 0.65	0.42 ± 0.76	42.8
Mature leaves	29	0.001 ± 0.003	0.007 ± 0.008	0.01 ± 0.008	1.0
Transparency film	0	0	0	0	0

[1]Six weeks × 20 trees.
[2]Twenty trees, data summed across weeks.
[3]Trees with no egg masses not included in means or SDs.
[4]Each tree contained one of each substrate and an estimated 198.4 leaf pairs.

TABLE 7. *DIAPREPES ABBREVIATUS* LARVAL TRAPPING BY ARTIFICIAL SUBSTRATE TYPES.

Substrate	Egg Masses = n[1]	Number Escaped	% Trapped
Wax paper	23	21	8.7
Freezer paper (plastic in)	18	18	0
Freezer paper (plastic out)	20	7	65.0
Transparency film	20	20	0
Parafilm M	23	23	0

[1]Five females and 1 male per replicate.

paper over 2.5 cm wide paper (Table 3, #9). No preference was expressed by females among the attractants attached to oviposition sites (Table 4).

Comparison of the various oviposition media outdoors under both field and semi-controlled conditions yielded similar results to those obtained in the laboratory. Whether tested with caged containerized red grapefruit trees or with planted 3-year-old 'Minneola' tangelo trees, freezer paper and wax paper were preferred over either mature leaves or transparency film (Tables 5 & 6). Transparency film was the least preferred as an oviposition site in both outdoor experiments.

When oviposition preference was examined in the caged, containerized grapefruit trees outdoors neither wax paper nor plastic side out freezer paper allowed neonate larvae to escape 21 days post oviposition. When strips were separated for examination, desiccated neonate larvae were observed. When rates of emergence from artificial substrates were examined in the laboratory, only plastic side out freezer paper, could be considered to have trapping ability (65% completely trapped, Table 7). The other substrates allowed almost a complete escape of neonate larvae (Table 7).

DISCUSSION

There were no statistical differences in egg masses deposited between media in the attractant experiment (Table 4). Clear oviposition preferences for mature leaves over immature and other media over mature leaves are presented in Table 3. The ovipositional preference of *D. abbreviatus* for mature leaves has been previously reported (Fennah 1942). This behavior by the weevil in selecting mature leaves for oviposition may increase its survival rate by avoiding insect feeding sites and leaf expansion.

Fennah (1942) reported a range of 0.25-0.73 egg masses per female per day when presenting paper strips to 30 females. In experiment 5, we presented a total of 375 females with an overnight opportunity for oviposition on artificial substrate and collected a mean of 0.58 egg masses/female/day (Table 3) which is comparable to Fennah (1942). The composition of the paper strips used by Fennah was not described. The neonate trapping ability of freezer paper (plastic out) was 65% and agrees with Fennah (1942) (Table 7). In the experiment performed outdoors using weathered artificial substrates (Table 6), egg masses were examined for evidence of larval mortality. Both wax paper and plastic side out freezer paper restricted neonate emergence from the egg mass. Wax paper, when weathered for several days, lost its hydrophobic coating and attained a more fibrous surface which appeared to be similar to freezer paper. Wolcott (1933) reported that thin, tough wrapping paper (unspecified) was effective in trapping larvae for three months in the tropics.

TABLE 8. *DIAPREPES ABBREVIATUS* EGG MASSES ON 'MINEOLA' TANGELO TREES IN THE FIELD BY WEEK.

Week	Trees with[1] No Egg Masses (%)	Trees w/Masses on Media, Not on Leaves (%)	Trees w/Masses on Leaves, Not on Media (%)
1	15 (75)	0 (0)	4 (20)
2	13 (65)	1 (5)	4 (20)
3	14 (70)	2 (10)	3 (15)
4	17 (85)	0 (0)	3 (15)
5	15 (75)	1 (5)	4 (20)
6	18 (90)	0 (0)	2 (10)

[1]Six trees had no eggs on media or leaves for the entire 6 weeks.

When freezer paper was presented for oviposition outdoors simultaneously with other artificial substrate types in young, caged grapefruit trees, it was preferred more than 3 to 1 over mature leaves (Table 5). However, this preference ratio increased to 50 to 1 in the field evaluation (Table 6). Overall, the field data in Table 6 indicate that 4 freezer paper strips/tree or about 5 wax paper strips/tree should receive the same number of egg masses as a tree with 198 leaf pairs suitable for oviposition. This is a calculated estimate, however, and should be tested in the field.

Our field experimental design took into consideration the caged tree data (Table 5) and logistics. According to the cage data, about 66 strips per tree would have allowed equal choice by a female in a field test. With an estimate of 198 leaf pairs suitable for oviposition, festooning a tree with oviposition strips was logistically not possible. Although the order of preference in Table 5 was similar to the field preference (Table 6), the cage preference for freezer paper was about 3:1, leaf:freezer paper (Table 5).

Table 8 gives weekly *D. abbreviatus* oviposition in the field. Over the 6 weeks of the trial, 65-90% of the trees had no egg masses deposited between either leaves or strips. Additionally, 10-20% of the trees had eggs masses deposited only between leaves, but not between the artificial sites in any week. In order to compare leaves and strips as oviposition sites in the field, it would be ideal if some egg masses were deposited between leaves and some between strips on the same tree. In only 3 weeks and on 3 trees did this situation occur.

In conducting the field experiments, about 10 min per tree were required to accurately search for egg masses. Spending ten minutes per tree is unrealistic for a grower to examine leaves for egg masses. If a monitoring method could be developed based on oviposition, it might serve as a tool for detection of *D. abbreviatus* prior to heavy infestation levels. An added benefit of freezer paper strips in this role is neonate larvae trapping ability. The only monitoring tools available to growers are wire mesh emergence traps and Tedders traps (Tedders & Wood 1994) both of which detect adult *D. abbreviatus* emerging from the soil, and visual detection of adults. These methods apparently detect an infestation one or more years after the initial infestation of a citrus grove.

Our data support the further investigation of freezer paper strips as a substrate for oviposition stimulant, as a monitoring tool, and a prototype oviposition trap for *D. abbreviatus.* Host oviposition preferences, oviposition stimulants, and general oviposition behavior of *D. abbreviatus* have not been studied. We believe study of these subjects could be beneficial for the management of this weevil in commercial agriculture.

Endnote

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