
Short Communication

Feeding Experience Enhances Attraction of Female *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) to Food Plant Odors

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INTRODUCTION

Learning in insects is broadly defined as a temporary modification of behavioral responsiveness as a result of experience (Papaj and Prokopy, 1989). According to Papaj and Prokopy (1989) and Thorpe (1993), a decline in a response in the absence of continued experience is another criterion that can be used to specify learning in insects. Learning in phytophagous insects may be manifested as altered responses to host plants following some type of experience. Individual insects exposed to different foods tend to select the food they most recently accepted and experienced, compared to novel foods (Papaj and Prokopy, 1989). Previous experience with a plant or its odor affects the behavior of many adult insects, such as *Battus philenor* (L.) (Papaj, 1986a,b), *Leptinotarsa decemlineata* (Say) (Visser and Thiery, 1986), and *Trichoplusia ni* (Hubner) (Landolt and Molina, 1996). In the pipevine swallowtail butterfly *B. philenor*, the positive effect of exposure to one food plant species on food acceptance was reversed by exposure to a second food plant species (Papaj *et al.*, 1986a).

Diaprepes abbreviatus is a polyphagous insect that feeds on foliage of at least 76 plant species (Martorell, 1976). The weevils are attracted to odors of food plants (Beavers *et al.*, 1982; Harari and Landolt, 1997) and form aggregations on or near new growth of host trees (Beavers *et al.*, 1982). Aggregations

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appear to result from orientation responses to odors from damaged plant foliage, weevil frass, and weevil pheromones (Harari and Landolt, 1997). These weevils may live for several months and their aggregations may persist on the same trees for weeks at a time (Beavers *et al.*, 1982; Jones and Schroeder, 1984).

In these studies we asked (1) Do female *D. abbreviatus* prefer a food they have experienced previously? and (2) Do encounters with a new food alter weevils' food preference? Answers to these questions may assist our understanding of how these weevils locate and select host plants and form mixed sex aggregations.

MATERIALS AND METHODS

Weevils

Adult *D. abbreviatus* were collected from ornamental black olive trees (*Bucida buceras*), also known as oxborn bucida, in Homestead, Dade County, FL, on June 6, 1996, and were sorted by sex in a laboratory (Harari and Landolt, 1997). Females were held in Plexiglas frame cages (30 × 30 × 30 cm) covered on five sides with 1-mm-mesh screening and a Plexiglas bottom. Two Parafilm sheets (3 cm wide × 10 cm long) were attached with adhesive tape to an inside wall of the cages to provide females with an oviposition substrate. Cages of female weevils were provided green beans or citrus foliage as food, depending on the experimental setup and were kept within large (3 × 3 × 3-m) field cages, 50 m apart, on the grounds of the USDA-ARS laboratories in Gainesville, FL (May–October).

Bioassay Setup

The attraction responses of females to odors of food materials were tested in two Y-tube olfactometers as described by Harari *et al.* (1994) and Harari and Landolt (1997). Each olfactometer was made of a Y-shaped glass tube. The glass tubing possessed a stem that was 2 cm i.d. × 14 cm long and two arms that were 2 cm i.d. × 10 cm long. Each arm of the Y led to a spherical glass trap, followed by a glass bulb containing a source of odors. Filtered (with charcoal), metered air was pushed into the system at 350 ml/min, first through the glass bulbs containing the test materials (food plant), then into each arm of the olfactometer, and, finally, through the stem of the olfactometer.

Young citrus foliage and green beans were selected for these experiments because *D. abbreviatus* feed on and are attracted to odors of both plant species (Beavers *et al.*, 1982, Harari and Landolt, 1997) and both have been used successfully in an insectary (USDA, ARS, Orlando, FL) as food for reproducing *D. abbreviatus* weevils. As an assay protocol, weevils were released individually at the base of the stem of the Y and their movement upwind toward an odor

source or control was observed. A positive response was recorded when a weevil entered one of the arms of the Y and fell into a spherical glass trap. No response was recorded and the assay was ended if a weevil did not reach the trap within 10 min of release. After the testing of five females to a treatment and control setup, the olfactometer was turned 90° to control for any position effects and the assay was repeated. Between assay replicates, glassware was washed with hot soapy water followed by ethyl alcohol.

In the first experiment we conducted two tests for effects of feeding experience on female *D. abbreviatus* attraction to two food plant odors, comparing attraction responses of weevils that were experienced versus weevils that were inexperienced with a particular food plant. One group of females was provided green beans as food for 7 days prior to the experiment and a second group was provided new-growth foliage of orange trees as food for 7 days prior to the experiment. Beetles were then held without food for 24 h prior to testing. Five females that had been fed citrus foliage were tested for attraction to odors of five citrus leaves versus the control (no food) and five females that had been fed citrus foliage were tested for attraction to two cut green beans versus the control (no food). This assay comparison was repeated 30 times, using the Y-tube olfactometer. Then five females that had been fed green beans were tested for attraction to odors of green beans versus the control and five females that had been fed green beans were tested for attraction to five citrus leaves versus the control. This assay comparison was also repeated 30 times using the Y-tube olfactometer (Table I).

The second experiment tested for reversal of effects of experience on weevil host attraction. That is, we determined if weevils respond preferentially to the host plant they encountered last rather than to any host plant previously encoun-

Table I. Comparison Sets of Treatments Using a Y-Tube Olfactometer

Pretreatment ^a	Source 1	Source 2	No. weevils ^b
Experiment 1			
Beans	Beans	Empty	147
Beans	Citrus leaves	Empty	130
Citrus	Beans	Empty	129
Citrus	Citrus leaves	Empty	133
Experiment 2			
Beans 1st/citrus 2nd	Beans	Empty	117
Beans 1st/citrus 2nd	Citrus leaves	Empty	70
Citrus 1st/beans 2nd	Beans	Empty	75
Citrus 1st/beans 2nd	Citrus leaves	Empty	69

^aFood types experienced by female *D. abbreviatus* prior to testing in the olfactometer.

^bNumber of weevils in an experiment that responded within 10 min, of 150 tested.

tered. To accomplish this, one group of weevils that had been fed green beans was switched to citrus foliage 7 days prior to the experiment, and another group of weevils that had been fed on citrus foliage was switched to green beans 7 days prior to the experiment. Weevils were then held for 24 h without food before testing. Comparisons were made of the attraction responses of these females to citrus foliage and to green beans. First, females that were first fed citrus foliage and then switched to green beans were tested in the Y-tube olfactometer for attraction to odor of five cut citrus leaves versus the control or to odor of two cut green beans versus the control. Then females that were first fed green beans and then switched to citrus foliage were tested for attraction to odor of green beans versus the control and to citrus leaves versus the control (Table I).

Statistical Analyses

The results of the two experiments were analyzed as two-choice data. The choice of one arm was compared with the choice of the other arm after nonresponsive individuals were omitted from the calculation. *G* statistics (Sokal and Rohlf, 1969) were used for replicates. Tests of goodness of fit were performed for the heterogeneity of the replicates and for the pooled data. Both tests were used to compute the significance of deviation from the expectation (1:1) for each treatment. The heterogeneity of the replicates of both experiments was not significant in any treatment ($P > 0.05$) (Table II).

Table II. Response of Female *D. abbreviatus* to Different Food Sources After Experiencing One Type of Food (Experiment 1) and After Experiencing Two Types of Food (Experiment 2)

Pretreatment ^a	Total ^b	Source 1	Attracted	Source 2	Attracted	$G_h^c - G_p^d$	G_t^e	df (G_t)
Experiment 1								
Beans	147	Beans	64.6%	Empty	35.4%	1.52	12.76	5*
Beans	130	Citrus	26.9%	Empty	73.1%	0.70	11.27	5*
Citrus	129	Beans	32.6%	Empty	67.4%	0.37	16.03	5*
Citrus	133	Citrus	64.7%	Empty	35.3%	0.78	11.61	5*
Experiment 2								
Beans 1st/citrus 2nd	117	Beans	35.9%	Empty	64.1%	1.82	9.44	5*
Beans 1st/citrus 2nd	70	Citrus	68.6%	Empty	31.4%	2.71	9.89	5*
Citrus 1st/beans 2nd	75	Beans	69.3%	Empty	30.7%	0.91	11.51	5*
Citrus 1st/beans 2nd	69	Citrus	29.0%	Empty	71.0%	1.21	12.58	5*

^aFood types experienced by female *D. abbreviatus* prior to testing in the olfactometer.

^bTotal number of tested females that responded.

^c*G* test for heterogeneity.

^d*G* test, pooled data.

^e*G* test, total.

* $P < 0.05$.

RESULTS

In the first experiment females that were previously fed green beans were significantly attracted into the arm of the Y-tube carrying odors of green beans compared to the arm carrying purified system air (control) but were not significantly attracted into the arm of the Y-tube carrying odor of citrus foliage compared to system air. Female weevils that were previously fed citrus leaves were significantly attracted to odor of citrus foliage compared to system air but were not attracted to odor of green beans compared to system air (Table II). In the second experiment the food that was provided to weevils was changed 7 days before assays. Female weevils that were fed on green beans first and on citrus leaves second were significantly attracted to odor of citrus leaves compared to system air but not significantly attracted to odor of green beans compared to system air. Females that were fed first on citrus leaves and second on green beans were significantly attracted to odor of green beans compared to system air but were not significantly attracted to odor of citrus leaves compared to system air (Table II).

DISCUSSION

Our experiments demonstrated that female *D. abbreviatus* responses to different food odors was affected by their prior feeding. This suggests that host plant preference, expressed as orientation, is affected by the type of plant the weevils fed on previously. Females were attracted by odors of the plant material (green beans or citrus leaves) on which they had fed prior to the experiments but not to a novel host plant material. Furthermore, this preference was altered after weevils fed on a second host plant material, by a subsequent preference in orientation to the second plant material, the last one fed upon. The results of the first experiment, in which females were more attracted to the plant material they had fed on, indicates that learning occurred, following the definition of Papaj and Prokopy (1989), Alcock (1993), and Cortesero *et al.* (1995). Therefore, we conclude that female *D. abbreviatus* are capable of learning host odor during feeding on that host plant. The results of the second experiment, in which females altered their food preference as a result of feeding on a second novel plant material, support this conclusion and are also in agreement with the definition of Papaj and Prokopy (1989) and Thorpe (1993) that a decline in a response in the absence of continued experience is a criterion to specify learning.

The olfactory system of phytophagous insects may monitor an array of odors that are encountered during foraging. The associative occurrence of the odors with different food resources are perceived and the linkage is learned. Linked odors then become cues for seeking the food resource (Lewis and Takasu, 1990). Prior experience (contact, feeding, oviposition) with a host plant is known

to enhance upwind orientation to odors of that host plant in other insects, such as adult *L. decemlineata* (Visser and Thiery, 1986), larval *Schistocerca* (Lee *et al.*, 1987), the parasitic wasp *Microplites croceipes* (Lewis and Takasu, 1990), and cabbage looper moths, *T. ni* (Landolt and Molina, 1996).

Female *D. abbreviatus* preferred an experienced food type and altered their preference of food type after experiencing a new type of food. The novel food was not necessarily a better nutritional type of food, since the same results, the switching of preference, occurred with feeding on either plant material. The propensity to accept a novel source and to reverse the acceptance by exposure to other fruit species occurs in female Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann) (Cooley *et al.*, 1986; Papaj *et al.*, 1987).

The ability to learn from experience, and therefore to change behavior as a response to a certain experience, is useful if it helps an individual adjust to variable conditions (Alcock, 1993). Animals may gain useful flexibility by adjusting their diets to what is most available to them. Females of the pipevine swallowtail butterfly, *B. philenor*, for example, search consistently for the more abundant of the two *Aristolochia* host species (Papaj, 1986a). Concentrating on a search for the most abundant food species is less time-consuming and results in more efficient foraging. Using a search image should increase the ability of a forager to detect a food item following a recent encounter with that food type (Prokopy *et al.*, 1986). Also, species with highly varied diets might be exposed to poisonous foods. The ability to learn and concentrate on the most abundant host plant species might reduce the chances of encountering less abundant nonhost poisonous plant species. Prokopy *et al.* (1986) suggested that such a defense function is involved in the learning of host cues. Furthermore, rejecting a host plant following learning of a different host plant's cues may reduce the possibility that a female entering a patch of a rare host type would stay in that patch, and continue to search.

Learning to prefer the last suitable food plant species encountered should have a distinct adaptive value for *D. abbreviatus*. Adult weevils aggregate to feed and mate (Beavers *et al.*, 1982; Jones and Schroeder, 1984), and may remain on the same tree up to several weeks. Thus, the diet of these weevils may remain constant for a long time and may be altered only when the suitable host plant foliage in an area is exhausted. Then, the weevils may aggregate on a different food species, altering their diet for another long period of time. In that situation, individual weevils may increase their fitness by altering food preferences to the novel experienced food species as follows. (1) *D. abbreviatus* may benefit by adjusting their food preference to the most available food species at a time. Using certain search cues to aggregate on the selected food type may be the most efficient foraging method. (2) As a polyphagous insect with a varied diet (Martorell, 1976), *D. abbreviatus* may be exposed to poisonous plant species while searching for food. By concentrating on one food species at a time, on

which they feed and mate, the risk of encountering a poisonous food species is greatly reduced.

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