OLFACTORY RESPONSE BY *CARPOPHILUS HEMIPTERUS* (L.), *CARPOPHILUS MUTILATUS* (ER.), *UROPHORUS HUMERALIS* (F.) AND *HAPTONCUS LUTEOLUS* (ER.) TO VARIOUS PORTIONS OF CITRUS FRUITS (NITIDULIDAE, COLEOPTERA)

by

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Abstract--The behavioral response of the Dried-fruit beetle, Carpophilus hemipterus (L.), Carpophilus mutilatus (Er.), Pineapple beetle, Urophorus humeralis (F.) and Haptoncus luteolus (Er.) to the pulp and rind of navel orange and Lisbon lemon and to cold pressed citrus oil was noted in a double choice olfactometer test. The results showed that all but C. hemipterus demonstrated an attraction to orange pulp. The attraction to lemon pulp was shown by all except C. mutilatus. A repellence to orange rind was demonstrated by all species. All except H. luteolus showed repellence to lemon rind. None of the species showed a directed (toward or away from) response to cold pressed citrus oil.

INTRODUCTION

In the San Joaquin Valley of California, fruit crops such as fig, citrus, plum, raisin grape, nectarine and peach are sometimes infested by nitidulid beetles. The most common species found in association with the fruits are the Dried-fruit beetle, Carpophilus hemipterus (L.), Carpophilus mutilatus (Er.), Haptoncus luteolus (Er.) and the Pineapple beetle, Urophorus humeralis (F.). These beetles feed on the ripening fruits and may transmit spoilage microorganisms (Warner, 1959). According to the California Fig Institute, damage to figs by nitidulids in 1974 was estimated at $750,000-$1,000,000.00. Damage was not uniform throughout 16,000 acres (Klamm, personal communication). Particularly large populations were noted where citrus groves were near figs (Soderstrom, personal communication). Barnes (1952) suggested that the problem on figs might be aggravated by the availability of food offered by the citrus fruits throughout the year.

In canvassing for occurrence and numbers of nitidulid beetles, fig and navel orange fruits have been used in bait traps.[1] The occurrence of large numbers of the four listed species indicates an ability of the insects to detect and orient to the fruit. The orientation seemed most likely to be an olfactory response to volatile compounds given off by the fruit. Several coleopterans have been shown to be attracted or repelled by fruit odors. The rice weevil, Sitophilus zeamais (Motschulsky), is attracted to acidic and neutral fractions of rice wheat and corn grains (Ohsawa et al., 1970) and the cotton boll weevil, Anthonomus grandis (Boheman), is attracted to such chemical fractions of the cotton bud as rose oxide, fenchone, menthone and isovaleraldehyde (Gueldner et al., 1970). The bruchid, Callosobruchus maculatus (F.), is repelled by oils derived from the rinds of 8 different citrus fruits (Su et al., 1972). To determine if the nitidulid beetles showed olfactory orientation to citrus, a laboratory study utilizing a double choice olfactometer was instigated.

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[1]Gary Obenauf, "Trap Design and Attractants: Report of Nitidulid and Drosophila Research" (California: Fig Institute, 1973), p. 15.

MATERIALS AND METHODS

A successful method of rearing the species was worked out by Soderstrom and Armstrong (U.S.D.A., Stored Product Insects Research Laboratory, Fresno, California). Four hundred ml of soil were mixed with 200 ml vermiculite (calculated volumes) in a one quart jar by closing the jar and shaking vigorously for 10-15 seconds. One hundred ml water were added to each jar containing the soil vermiculite mixtures. The jar was then autoclaved at 132°C. and 15 p.s.i. for 30 minutes and allowed to cool overnight. The food media consisted of processed extra fancy grade figs which had been soaked in tap water for 24 hours before use. Initially three figs were placed on the soil surface in the culture jar. For each species cultured, 30 adults were enclosed in each jar with a filter paper and brass screen lid. Cultures are held for not more than three consecutive generations with an addition of water-soaked figs as necessary. The cultures were held in a room at 27 $\pm$ 1°C. and 60 $\pm$ 5% RH. until the beetles were required for the research program. The progeny from the stock cultures were used in all tests.

The olfactometer design (Figures 1 and 2) is of a large H with a release tube attached to the crossbar tube. The body of the H is constructed of glass tubing with an interior diameter of 1.5 cm. The lateral tubes are 25 cm in length and to their ends are attached a 12 cm long collecting chamber with a diameter of 1.5 cm. The mid points of the lateral tubes are connected with a 57 cm long crossbar tube which at its middle has a perpendicular release tube of 47 cm in length. The last 8 cm of the release tube is above the plane of the rest of the olfactometer.

The collecting chambers are designed for the convenience of cleaning, the placement of an odor source, the entrance of a stream of air to pass through the chamber and into the body of the olfactometer. The treated material was inserted into one of the collecting chambers and the other three chambers remained empty.

Before use and between trials, the olfactometer was washed with water, rinsed with acetone, and dried for fifteen minutes by blowing 40°C. air into the release tube. The cleaning was to remove any residual odors and debris.

In order to obtain uniform conditions within the olfactometer, it was placed in an environmental chamber of 183 x 183 x 183 cm which was maintained at a temperature of 27 $\pm$ 1°C., which is optimal temperature for the dried fruit beetle (Lindgren and Vincent, 1953), and a relative humidity of 60 $\pm$ 5%. The olfactometer was placed in a 99 x 68 x 19 cm flat black box to reduce light reflections. The entire unit was exposed for one hour to equalize the interior environment with that of the room before introduction of the beetles. During the study a 15 watt fluorescent lamp, placed 45 cm above the olfactometer, gave continuous illumination.

The air passing through the flowmeters (B) at 50 ml per minute traversed the collecting chambers (A), the lateral tubes, the crossbar tube, the release tube and finally the air exhaust (Figure 1).

In this olfactometer a beetle reaching the treated chamber passed two points of choice: one at the outlet of the releasing tube, the other at the junction of the lateral tube which had a treated and untreated collecting chamber at each end. The beetles going away from the treated side had only a single choice.

The portions of the citrus fruit, selected for use as odor sources, were the rind and the pulp of both navel orange and Lisbon lemon. The cold pressed citrus oil obtained from V. P. Maier (U.S.D.A., Fruit and Vegetable Chemistry Laboratory, Pasadena, California) was also tested. The orange or lemon was briefly washed in 80% ethyl alcohol, brushed to remove adhering dirt, rinsed in water, washed in detergent; rinsed, washed again; and finally rinsed in deionized water. The surface was then blotted with sterilized paper. To acquire the rind only, the surface of the rind was scraped with a clean knife onto a preweighed, sterilized absorbent piece of cotton that would fit into a collecting chamber. Using a knife, cleaned before each operation, the pulp was obtained by carefully peeling the rind away and cutting the pulp into 0.5 cm cube which was placed on cotton as above. The cold pressed citrus oil was applied to the cotton with a microliter syringe. The amounts used per replicate were: orange rind 39 mg, lemon rind 44 mg, pulp of either 20 mg and the cold pressed citrus oil 1 ml.

To start a replicate, treated cotton was inserted into a collecting chamber and 15 adult beetles, randomly taken from the culture jar, were placed in the release chamber. Thirty minutes after the release time, the individuals that had entered a collecting chamber were counted. The numbers were recorded for each of the following designated chambers:

treated, same side, opposite from, or diagonally from the treated. As the numbers in the nontreated chambers showed no statistical differences, they were totaled, averaged and compared with the mean number found in the treated chamber. The beetles remaining in the release chamber or in the cylinders between the collecting chambers were designated as nonparticipants in the experiment. For each species, the trials were replicated 10 times and 150 individuals were tested.

An assumption was made that, if there was no specific orientation, the numbers in each collecting chamber would be equal. If more than the expected number occurred in the treated chamber, the response would be said to be due to an attraction; if less, the response would be considered to be due to a repellence to the odors emitted from the test materials. Data from the bioassays were analyzed by analysis of variance and F test.

RESULTS

In all the tests, 3000 individuals were used, but only 1833 individuals participated. All the species, except C. hemipterus to orange (36% in treated chamber) and C. mutilatus to lemon (35% in treated chamber), were found to be attracted in sufficient numbers to odors emanating from the pulp. However, all species, except H. luteolus to lemon rind, showed a significant avoidance or repellence to the odors emanating from citrus rind (Table 1). The high figure, 31%, for H. luteolus captured at the lemon rind, must be tempered with the large number of nonparticipating individuals (Table 1). None of the species showed a directed (toward or away from) response to the odors from the cold pressed citrus oil (Table 1).

DISCUSSION

The oriented movements to air which flows over a source, citrus pulp or rind (Table 1), demonstrates the ability of the nitidulid beetles, associated with figs, to recognize and respond to olfactory clues. The data also substantiate the differential attraction that various fruits may have for any given species as shown by the 60% of _H. luteolus_ and 55% of _U. humeralis_ responding to lemon pulp whereas only 41% and 37% responded to the same amount of orange pulp (Table 1). The demonstrated attraction verifies the use of fruits as a bait for detection or survey and suggests the possibility that, given sufficient dispersal, odors might be useful in control.

The avoidance of the lemon rind, _C. mutilatus_ 0%, _U. humeralis_ 3%, and _C. hemipterus_ 7% and orange rind, _U. humeralis_ 2%, _C. mutilatus_ 4%, _C. hemipterus_ 5% and _H. luteolus_ 9% was striking. Further study of the repellence to citrus rind or the active fraction thereof might demonstrate control possibilities.

The lack of response to cold pressed citrus oil was surprising since an oil from the epicarp of the citrus peel proved highly toxic to the cowpea weevil, _Callosobruchus maculatus_ (Su et al., 1972). However, the concentration of cold pressed citrus oil used in the experiment might be lower than the recognition threshold of the nitidulid beetles.

The data suggest the use of citrus pulp alone would be more effective than cut citrus with attached peel which is the current practice (Obenauf, 1973; Armstrong, personal communication). The sequential and simultaneous occurrence of a repellent and an attractant (Maxwell et al., 1963) with the latter overcoming the former (Dethier, 1947) appears to apply also to citrus fruits. If the pulp can overcome the apparently stronger repellence, then its use in control may not be feasible.

Further research should be conducted to extract, purify and identify the active fraction of citrus rind or pulp, to study the beetles' response to different concentrations of the fruit products, and to denote changes in response due to variations in temperature, humidity or daylight.

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APPENDIX

Table 1. Response of Nitidulid beetles to various portions of citrus fruit odors in a double choice olfactometer (Figure 1).

Species	Average number and percentage[a] of adults from collecting chambers and average number of non-participating					F test	Significance *=at p0.05 **=at p0.01 NSD=non-significance
	Treated		Untreated[b]		Nonparticipants		
	No.	%	No.	%	No.		
Orange pulp							
Carpophilus hemipterus (L.)	3.5	36	2.0	21.3	5.3	2.87	NSD
Carpophilus mutilatus (Er.)	4.6	42	2.1	19.3	4.1	8.94	**
Urophorus humeralis (F.)	4.6	41	2.2	19.6	3.7	5.45	**
Haptoncus luteolus (Er.)	2.5	37	1.4	21	8.3	3.78	*
Lemon pulp							
C. hemipterus	4.5	51	1.5	16.3	6.1	16.48	**
C. mutilatus	3.3	35	2.0	21.6	5.6	2.45	NSD
U. humeralis	4.8	55	1.3	15	6.2	11.39	**
H. luteolus	5.0	60	1.1	13.3	6.7	22.94	**
Orange rind							
C. hemipterus	0.5	5	3.4	31.6	4.2	19.70	**
C. mutilatus	0.4	4	3.3	32	4.7	27.45	**
U. humeralis	0.2	2	3.3	32.6	4.8	12.46	**
H. luteolus	0.8	9	2.8	30.3	5.9	7.39	**

Table 1 (continued)

Species	Average number and percentage[a] of adults from collecting chambers and average number of non-participating					F test	Significance *=at p0.05 **=at p0.01 NSD=non-significance
	Treated		Untreated[b]		Nonparticipants		
	No.	%	No.	%	No.		
Lemon rind							
C. hemipterus	0.5	7	2.6	31	7.5	9.28	**
C. mutilatus	0	0	3.1	33.3	5.7	24.02	**
U. humeralis	0.3	3	3.0	32.3	5.7	11.26	**
H. luteolus	1.3	31	1.0	23	10.8	1.89	NSD
Cold press citrus oil							
C. hemipterus	1.6	17	2.6	27.6	5.7	2.8	NSD
C. mutilatus	1.4	13	3	29	4.4	3.04	NSD
U. humeralis	1.9	16	3.3	28	3.1	1.25	NSD
H. luteolus	0.9	13	2.0	29	8.2	2.47	NSD

[a]average of 10 replicates.

[b]average of adults taken from the same side, opposite or diagonal from the treated chamber.

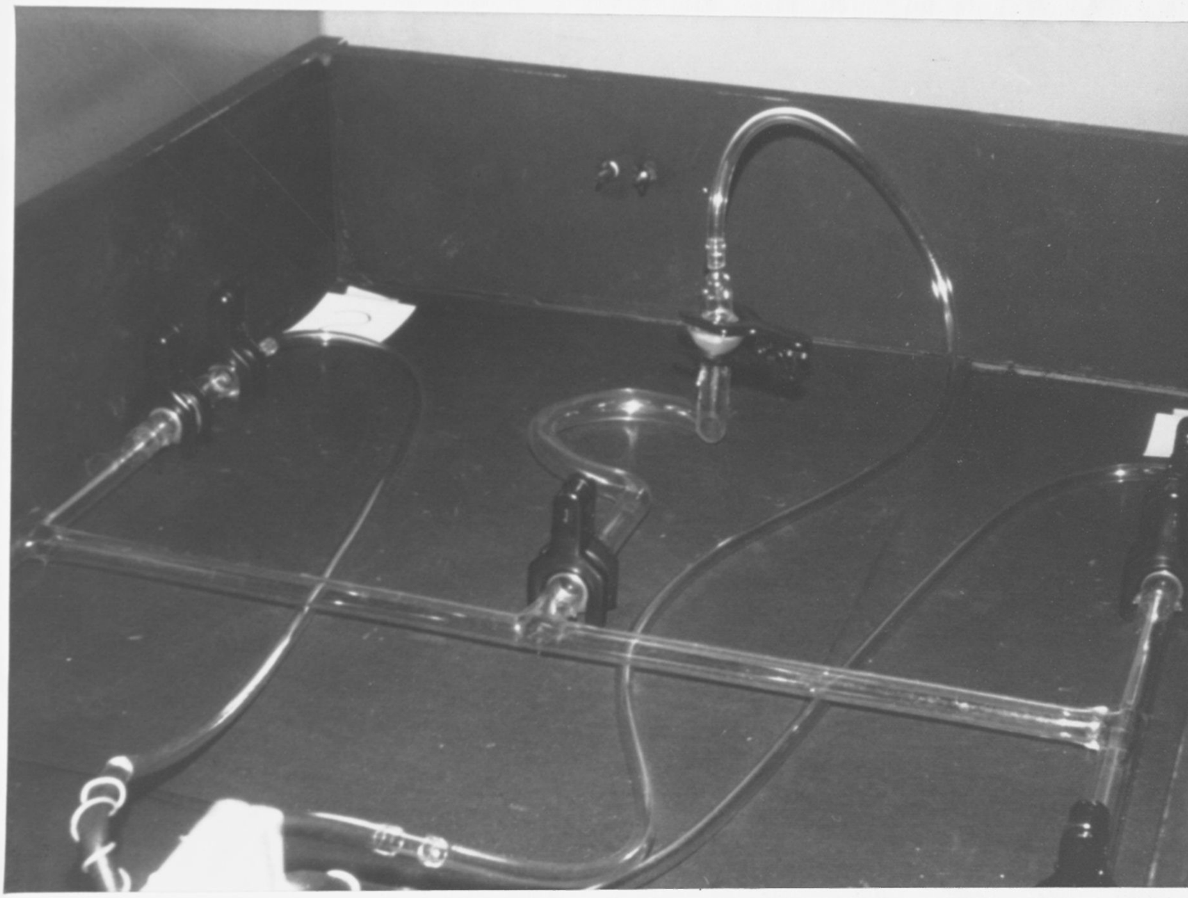

Figure 2. Photograph of the double choice olfactometer for the bioassay of food attractants and repellents used in the study of Nitidulid beetle response to various portions of citrus fruit.