FIELD TESTS FOR AN ADULT WESTERN CORN ROOTWORM AGGREGATION PHEROMONE ASSOCIATED WITH THE PHAGOSTIMULATORY CHARACTERISTIC OF BITTER CUCURBITA SPP.

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Abstract: In field tests, there were no significant differences in the number of adult western corn rootworms, *Diabrotica virgifera virgifera* LeConte captured on sticky traps baited with freshly sliced *Cucurbita foetidissima* fruit, traps baited with dried, finely-ground *C. andreana* × *C. maxima* fruit, and unbaited controls. Further, there was no effect due to the presence of beetles actively feeding on either of the test materials. The data suggest that no aggregation pheromone nor increased attractiveness of bitter cucurbit fruit result from such an interactive relationship.

Key Words: Western corn rootworm, *Diabrotica virgifera virgifera*, cucurbitacin, *Cucurbita* pheromone.


The plant family Cucurbitaceae contains approximately 900 species in 100 genera. Of these, at least 100 species in 30 genera have been shown to contain cucurbitacins. Chemically, cucurbitacins are a group of about 20 oxygenated tetracyclic triterpenes. It is the cucurbitacins which are responsible for the characteristic bitter taste associated with most wild cucurbits. The extremely bitter taste of pure cucurbitacins in conjunction with their high mammalian toxicity suggest that cucurbitacins function primarily as a plant defense mechanism in the bitter cucurbits (Metcalf et al. 1980).

The association between *Diabrotica* beetles and *Cucurbita* spp. plants has long been recognized. Contardi (1939) first described the compulsive *Diabrotica* beetle feeding behavior elicited by bitter cucurbits. Since that time numerous authors, including Chambliss and Jones (1966), and Metcalf et al. (1980), have investigated this relationship. Rhodes et al. (1980) considered the accumulation of western corn rootworm (WCR) adults (*Diabrotica virgifera virgifera* LeConte) on exposed, sliced fruits of the cucurbit hybrids *Cucurbita andreana* × *C. maxima* and *C. texana* × *C. pepo* as evidence of WCR oriented movements. They identified WCR as being “attracted” to the bitter cucurbit fruits. Metcalf et al. (1980) observed that cucurbitacins were responsible for arresting searching behavior and producing compulsive feeding by *Diabrotica* beetles. They further suggested that the relatively nonvolatile cucurbitacins may co-distill with water vapor from the plant to act as a true attractant. Pure cucurbitacin was characterized as a phagostimulant but not as an attractant to WCR by Howe et al. (1976). They attributed the lack of any attractant response by WCR to pure cucurbitacin to the low volatility and odorless characteristics of highly purified cucurbitacin. In attempting to account for the rapidity with which large aggregations of western

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corn rootworms appear on bitter *Cucurbita* spp., Howe et al. (1976) suggested that while feeding on bitter cucurbits, the WCR may be induced to emit an extremely potent aggregation pheromone.

In both laboratory and field tests with slices or homoginates of fresh *Cucurbita* spp. fruits, Branson and Guss (1983) could detect no significantly different olfactory responses of WCR between bitter or nonbitter cucurbits. Further, in field tests, unbaited traps and traps baited with either bitter or nonbitter cucurbits captured similar numbers of WCR and northern corn rootworms (*Diabrotica barberi*, Smith and Lawrence). They did not, however, test for an aggregation pheromone or other type of increased attractancy due to beetles actively feeding on bitter cucurbits.

The objective of our experiments was to bioassay for the existence of a WCR aggregation pheromone or other increased attractancy produced as a result of beetles actively feeding on bitter cucurbits. Also, we conducted tests to evaluate the olfactory response of WCR to fresh slices of bitter wild buffalo gourd, *C. foetidissima*, fruits.

**MATERIALS AND METHODS**

To assess the attractancy of *C. foetidissima* to WCR adults, we used methods similar to those described by Branson and Guss (1983). Trapping sites consisted of corn stalks stripped of leaves and cut off ca. 1 m above the soil surface. Traps were attached to the top of these corn stalks. Traps consisted of a 473 cm\(^3\) paper carton with 24, 1-cm Diam holes punched in the side of the carton. A slice (ca. 35 g) of freshly-cut fruit was isolated in each carton by a wire-screen container. The outside of each paper carton was coated with Stikem\(^{\text{®}}\). Two experiments, each comparing baited to unbaited traps, were conducted. The first test (seven replications) ran from 27 to 31 August 1983, while the second test (five replications) ran from 29 to 31 August 1983. Captured beetles were counted and removed at ca. noon each day in both experiments.

The following trapping methodology was devised to bioassay for increased attractancy due either to emission of an aggregation pheromone or interaction between actively feeding beetles and bitter cucurbits. As in the previous experiment, sites consisted of a corn stalk stripped of leaves and cut off ca. 1 m above the soil surface. A 473 cm\(^3\) paper carton with a hole cut in the top was slipped onto the top of the corn stalk. A paper plate was wired to the top of the paper carton to provide a trap site platform in the corn field. Due to the strong arrestment response by beetles to bitter cucurbits, it was essential to avoid allowing direct contact of test beetles with the material to be evaluated. For this reason we created a double isolation system for the cucurbit materials tested. Each material tested was placed within a wire-screen cylindrical cage (13 cm tall × 7 cm Diam). The cage containing the test material was then placed within a larger wire-screen cylindrical cage (20 cm tall × 9.5 cm Diam). This double cage system, containing the test material, was then placed on top of the trapping site platform. A Pherocon\(^{\text{®}}\) AM sticky trap was placed around the corn stalk directly below the attractant site to monitor the number of WCR beetles attracted (Fig. 1).

Three separate tests, each consisting of primarily the same treatments, were conducted. The materials tested were: (1) a 35 g slice of fresh *C. foetidissima* fruit; (2) 50 WCR beetles feeding on a 35 g slice of fresh *C. foetidissima* fruit; (3) 4 g of
dried, finely-ground *C. andreana* × *C. maxima* fruit; (4) 50 WCR beetles feeding on 4 g of dried, finely-ground *C. andreana* × *C. maxima* fruit; (5) 50 WCR beetles alone; (6) an unbaited control. Unsexed beetles used in these tests were field-collected ca. 24 h prior to test initiation. The first test compared treatments 1, 2, 3, 4 and 5 over a 24-h sampling period from 8 to 9 August 1983. The second test, conducted from 11 to 13 August 1983, contained two, 24-h sampling periods, and included all six treatments listed above. The third test was conducted during 15-16 August 1983 and included treatments 1, 2, 3, 4 and 6. The first, second and third tests had four, six, and five replications, respectively.

As a validation of the trapping system described above, trap catches at sites baited with a known WCR attractant were compared to catches at unbaited sites. On 29 August we placed into a corn field, an experiment of three replications comparing unbaited traps to traps baited with WCR sex pheromone, as described by Guss et al. (1982). The pheromone was contained within slow release fibers provided by Albany International. Individual hollow fibers were 1.5 cm long and had an inside Diam of 200 µm. Fibers were sealed at one end to produce a
capillary reservoir from which pheromone was released at a precise and constant rate. Ten fibers were used in each baited trap.

All field tests utilized a completely randomized design. The number of replications varied from test to test but the spacing between trapping sites remained constant in all tests. Trapping sites were equally spaced within corn fields according to a gridwork, with each trap ca. 25 m from its nearest neighbor. All data were analyzed via standard ANOVA procedures.

RESULTS AND DISCUSSION

The objectives of this experiment were developed as a result of preliminary field observations on the potential attractiveness of bitter C. foetidissima fruits to field populations of adult WCR. In these initial tests, 2.5 cm thick, cross-sectional fruit slices from the central portion of gourds, which were about 10 cm in Diam, were wedged into leaf axils of corn plants (one per plant) approximately one meter above ground level. The fruit slices were left undisturbed for 48 h. Field populations prior to test initiation were approximately 4 beetles/plant. After 48 h the flesh of the fruit slices had been completely consumed, leaving only the tough inedible rind. Each corn plant which had a fruit slice placed on it had approximately 30 WCR adults moving about in a highly visible manner. This preliminary observation of highly visible aggregations of WCR adults could easily lead one to erroneously conclude a true attractant response had occurred, be it a response to fruit volatiles or an aggregation pheromone.

In field tests designed to evaluate the attractiveness of C. foetidissima to WCR we could not demonstrate any significant olfactory attractiveness (Table 1). These data, although for a different cucurbit species, support those reported by Branson and Guss (1983). Data presented in Table 1 were obtained from field tests which employed paper carton traps similar to those used by Branson and Guss (1983).

Table 1. Mean number of adult western corn rootworms caught/trap comparing paper carton traps baited with fresh Cucurbita foetidissima fruit to unbaited traps.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Days after initial trap placement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Unbaited</td>
<td>13.3</td>
</tr>
<tr>
<td>Baited with fruit slices</td>
<td>20.7</td>
</tr>
</tbody>
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*No significant differences were observed. (ANOVA, $P > 0.05$).

Field tests designed to assay the validity of our trapping methods in the aggregation pheromone experiments yielded the following results. Traps baited with slow release fibers containing WCR sex pheromone had an average catch of 125.7 beetles/trap while unbaited sites averaged only 19.3 beetles/trap. These results convinced us of the validity of this method.
With data collected from three separate field tests, we could not demonstrate any statistically significant olfactory response to either of the cucurbit materials tested or beetles feeding on such materials. Trap catches for sites baited with fresh *C. foetidissima* fruit or dried *C. andreana × C. maxima* fruit did not differ from unbaited trap catches. Further, there was no effect due to the presence of beetles actively feeding on either of the test materials (Table 2).

Table 2. Olfactory response of adult western corn rootworms to fresh *Cucurbita foetidissima* fruit slices and dried finely-ground *C. andreana × C. maxima* fruit.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean number of beetles caught/trap*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment number</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Fresh cucurbit</td>
<td>54.8</td>
</tr>
<tr>
<td>Fifty beetles feeding on fresh cucurbit</td>
<td>61.3</td>
</tr>
<tr>
<td>Dry cucurbit</td>
<td>54.7</td>
</tr>
<tr>
<td>Fifty beetles feeding on dry cucurbit</td>
<td>51.3</td>
</tr>
<tr>
<td>Unbaited control</td>
<td>67.6</td>
</tr>
<tr>
<td>Fifty beetles alone</td>
<td>59.5</td>
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</tbody>
</table>

*No significant differences were observed, (ANOVA, \(P > 0.05\)).

During the course of previous research into the various *Diabrotica* spp.-*Cucurbita* spp. interactions, investigators have frequently failed to design experiments capable of clearly delineating between attractant and arrestance responses by *Diabrotica* spp. Dethier et al. (1960) defined an attractant as a substance which causes insects to make oriented movements towards its source. The term attractant has often been used to describe the behavior of insects that are aggregated, regardless of the mechanism responsible for the aggregation. This is well illustrated in one of Dethier’s earlier papers (1955). In that study, Dethier documented that sugar, which had previously been termed an attractant when describing its effect on black blow fly behavior, actually acted in an orthokinetic manner to produce fly aggregations. Sucrose has a vapor pressure so low that it could not possibly have acted as an attractant. Flies which encountered sucrose during the course of normally occurring movement were induced to slow down or stop locomotor behavior (orthokinesis) primarily due to stimuli received as a result of direct contact with the sucrose. Dethier et al. (1960) described an arrestance as a chemical which, upon direct contact, causes insects to aggregate as a result of undirected kinetic reactions, either by slowing down or stopping locomotor activity (orthokinesis) or an increase in the rate of turning (klinokinesis). Our experiments were designed to test only for attractant responses by WCR adults.

WCR adults have been described as being attracted to bitter *Cucurbita* spp. fruit (Rhodes et al. 1980). In light of data presented herein, we can only conclude that, given the conditions of these tests, there is no WCR aggregation pheromone produced and also, there is no increased attractancy of bitter fruits due to any interactive relationship caused by actively feeding beetles. Fruits of bitter...
Cucurbita spp. are, without a doubt, potent phagostimulants and arrestants of WCR adults, but appear to have no significant olfactory attractiveness to WCR.

Several authors have suggested that the Diabrotica spp. - cucurbitacin interaction offers potential for ecologically sound pest management. It has been proposed that bitter cucurbit fruit may function ideally as either a bait in adult WCR population monitoring traps, or as one component of an attract and kill, poisoned bait program for adult WCR population suppression (Rhodes et al. 1980; Metcalf et al. 1982; Briggs and Shaw 1983). The lack of attractiveness of bitter cucurbit fruit to WCR as documented for C. texana and C. andreana by Branson and Guss (1983), and for fresh C. foetidissima and dried, finely-ground C. andreana × C. maxima reported within this paper, in conjunction with the lack of a WCR aggregation pheromone as reported herein, are facts which should be considered when future investigators design and interpret population monitoring trap counts or poisoned bait adult suppression programs.

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