Feeding and arrestment responses of *Diabrotica speciosa* to cucurbitacin-content formulations

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Abstract – The objective of this work was to evaluate arrestant and stimulant feeding effects on *Diabrotica speciosa* (Ger.), using cucurbitacin-content starch-based formulations prepared with varying starch sources, and adding potassium lignate. In a glass slide assay, the wash off resistance of formulations was compared. Potassium lignate did not improve wash off resistance. *Lagenaria vulgaris* L. powder, in which cucurbitacin B concentration was determined as 0.28%, was added to the most adhesive formulation. The resultant material was used in a two-choice assay in which leaves of common bean, *Phaseolus vulgaris* L., treated with concentrations of 2.5%, 5%, 10%, 15% and 20% were offered to insects together with untreated control leaves. Greater number of insects and leaves consumed were found on leaves treated with cucurbitacin-content formulation (2.5%, 5% – greatest response –, 10% and 15% concentrations) than on untreated control leaves. The concentration, in which responses were higher, was sprayed in a bean field at 1,000, 1,900 and 3,000 g ha^{-1}. Greater number of beetles was found in plots treated with the highest dosage, 3 and 6 days after spraying. Ten days after spraying, no significant differences were found among dosages, probably due to washoff of the bait.

Index terms: Insecta, Coleoptera, bait, semiochemical, rootworm.

Respostas alimentares e arrestantes de *Diabrotica speciosa* a formulações contendo cucurbitacina

Resumo – O objetivo deste trabalho foi avaliar os efeitos alimentares estimulantes e arrestantes para *Diabrotica speciosa* (Ger.), a partir de formulações amiláceas com cucurbitacina, variando-se as fontes de amido e adicionando-se lignato de potássio. Em ensaio com lâmina de vidro, comparou-se a resistência à lavagem das formulações. O lignato de potássio não aumentou a resistência à lavagem. Pó de *Lagenaria vulgaris* L., cuja concentração de cucurbitacina B foi determinada como sendo de 0,28%, foi adicionado à formulação mais adesiva. O material resultante foi utilizado em ensaios com dupla chance de escolha, nos quais folhas de feijão, *Phaseolus vulgaris* L., pulverizadas com concentrações de 2,5%, 5%, 10%, 15% e 20% foram oferecidas aos insetos juntamente com folhas não tratadas. Foram observados maior número de insetos e maior quantidade de folhas consumidas nas folhas tratadas com formulações com cucurbitacina (2,5%, 5% – maior resposta –, 10% e 15%) do que nas folhas não tratadas. A formulação cuja concentração teve maior resposta foi pulverizada em lavoura de feijão, nas dosagens de 1.000, 1.900 e 3.000 g ha^{-1}. O maior número de adultos de *D. speciosa* foi encontrado nas parcelas tratadas com a maior dosagem, aos 3 e 6 dias após a pulverização. Dez dias após a pulverização, não foram encontradas diferenças entre as dosagens, provavelmente por causa da lavagem da isca pelas chuvas.

Termos para indexação: Insecta, Coleoptera, isca, semioquímico, larva alfinete.

Introduction

Management tools for *Diabrotica speciosa* (Ger.) include mostly chemical insecticides. For adults control, chemicals are used several times per season due to the beetle reinfestation. Larvae feed on roots of plants such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), potato (*Solanum tuberosum* L.) and black oat (*Avena strigosa* Schreb.). Larvae management is also restricted to chemical control via seed treatment, which is less effective and cause soil pollution. The development of alternative tactics for pest management is a high priority throughout Latin America agricultural areas.

Diabroticite and Aulacophorite beetles (Luperini tribe) (Coleoptera: Chrysomelidae) feed compulsively and are arrested by cucurbitacins (Chamblis & Jones, 1966;

Howe et al., 1976). *D. speciosa* sequestration and storage of 23, 24-dihydrocucurbitacin D was reported (Nishida et al., 1986; Nishida & Fukami, 1990). This insect is highly responsive to cucurbitacin from *Lagenaria vulgaris* L. (Roel & Zatarin, 1989; Ventura et al., 1996), and is also attracted by 1,4-dimethoxybenzene, TIC (1,2,4-trimethoxybenzene + indole + trans-cinnamaldehyde) and VIP (veratrole + indole + phenilacetaldehyde) from *Cucurbita* spp. blossoms (Ventura et al., 2000). Interactions between Luperini beetles and cucurbitaceous plants show potential to develop suitable tools in integrated pest management programs (Metcalf & Metcalf, 1992; Deem-Dickson & Metcalf, 1995).

A successful strategy to manage *Diabrotica* spp. areawide in the United States is a semiochemical bait, which is effective, selective and allowing conventional insecticide dosage reduction (Chandler & Sutter, 1997; Chandler, 1998; Faust & Chandler, 1998).

The objective of this study was to assess the responses of *D. speciosa* to a specific leaf adherent bait containing starch and cucurbitacin (from *L. vulgaris*).

Material and Methods

The field experiment was carried out at Universidade Estadual de Londrina, School Farm, at 23º19'S, 51º12'W, in Londrina, Paraná, Brazil. Common bean, *Phaseolus vulgaris* L., cv. Pérola, was sown on September 17, 2001. The cucurbitacin-rich wild plant *Lagenaria vulgaris* and common bean were grown in greenhouse. Insects were field collected using a sweep net, and were fed in the laboratory with carrot slices and water.

Formulation procedures

Green fruits of *L. vulgaris* were collected, cut transversely (ca. 2 cm), dried in oven at 70ºC during 48 hours, and ground in a blender. Powdered fruits (100 g) were mixed with distillated water: 2-propanol (4:1) (2,000 mL). Formulations were prepared using starch alone, or in combination with potassium lignate prepared mixing kraft lignin with potassium hydroxide and deionized water (Tamez-Guerra et al., 2000). Starch sources were: corn (*Zea mays* L.) starch Maizena; cassava (*Manihot esculentum* Crantz) starch Zaeli, and cassava starch Pinduca. Starch and *L. vulgaris* powder were combined in equal parts. Additional procedures were carried out according to Tamez-Guerra et al. (2000).

Determination of cucurbitacin content in *L. vulgaris* samples

The standard stock solution of cucurbitacin B (0.32 mg mL^{-1}) was prepared by weighing out pure standard solution dissolved in ethanol. Standard solutions of cucurbitacin B were prepared by using suitable dilutions of stock solutions to obtain final concentrations between $2.05x10^{-3}$ mg mL^{-1} and $1.30x10^{-2}$ mg mL^{-1}. Absorbance measurements were performed using an Ocean Optics miniature fiber optic CHEM2000-UV-VIS spectrophotometer, equipped with a deuterium tungsten light source with an integrated curvette holder, a 300 μm solarization-resistant optical fiber. Absorbance values were recorded at 228 nm. Powdered fruits of *L. vulgaris* (2.5 g) were extracted in ethanol. The solution remained at rest for 48 hours at 8°C. The ethanolic extract was filtered using filter paper. The sample was prepared by diluting 1 mL of the resulting solution with ethanol in a 25 mL volumetric flask.

Adherence assay

To test formulations adherence a glass slide assay was adapted from McGuire & Shasha (1992). Formulation granules (20 mg) were sprinkled on a wet glass slide surface and air dried for 24 hours. Treated glass slide surface was moved back and forth 2 cm below a stream of water (40 mL at 20 mL min^{-1}) from a burette. Procedures were repeated for 4 days (n = 5). Formulation loss was determinated by weighing slides and subtracting from the initial weight.

Laboratory and field bioassays

The most adherent formulation was added to distillated water at concentrations 2.5%, 5%, 10%, 15% and 20%. Suspension was applied to leaves using a manual sprayer. Squares of 1.5x1.5 cm were cut from the bean leaves and placed in Petri dishes of 8.5 cm diameter. Two treated and two untreated squares were placed alternately on the edges of the dish. One beetle was released in the center of the arena. Feeding was measured 24 hours later. Images of leaves were digitized and fed area established using Siarcs software (Jorge, 1997). Insects were observed during the first 6 hours of the assay and recorded on the host where they were located. Preference index (PI) was calculated using $[(T - C)/(T + C)]*100$ (T is the eaten area or number of insects feeding on treated leaves; C is the eaten area or number of insects feeding on untreated control leaves (Escoubas et al., 1993).

In the field, 1,000, 1,900 and 3,500 g ha^{-1} of the formulation (in the concentration in which the highest response was found in the laboratory bioassays) were tested along with an untreated control (n = 5). Common bean plots were 6x3 m (0.45 m row width). *D. speciosa* population was estimated using 12 sweep net samples plot^{-1} at 3, 6 and 10 days after application. A pluviometer was used to measure rainfall during assessment periods.

Experimental design and statistical analysis

Laboratory tests were conducted in a completely randomized design, and the field assay, in a randomized complete block design. Paired-choice assays were compared using the paired-sample t-test, due to the lack of independence between treatments (Horton, 1995). For the field assay, analysis of variance (Anova) was performed on data, and Duncan's multiple range test was used to compare means (SAS Institute, 1989). Data were transformed log (x+1) constant to normalize the data and reduce heterogeneity of variances. Means and standard errors of means are presented for untransformed data.

Results and Discussion

Absorbance responses were linear in relation to the concentration of cucurbitacin B, over the range 2.05x10^{-3} mg mL^{-1} and 1.30x10^{-2} mg mL^{-1}. The regression equation obtained was A = 0.1317 + 3.39942c (where c is the concentration of cucurbitacin in mg mL^{-1}), and the correlation coefficient was r = 0.9987, indicating an excellent linearity. The concentration found in 0.1 g of powder of *L. vulgaris* was 0.2848 mg of cucurbitacin B.

Adherence of formulations was affected by ingredients in their compositions (F = 5.98; p<0.003). In cassava starch Pinduca granules percentage of loss was significantly lesser than corn starch and corn starch + potassium lignate granules (Table 1). The adherence of granules was not improved by addition of potassium lignate (Table 1).

Table 1. Effect of formulation ingredients on adherence of cucurbitacin content granules on a glass slide assay(1).

Formulation	Loss of granules from slides(1)
Corn starch Maizena	56.4 (±0.03)a
Corn starch Maizena + potassium lignate	60.0 (±0.03)a
Cassava starch Zaeli	43.6 (±0.04)abc
Cassava starch Zaeli + potassium lignate	49.2 (±0.03)abc
Cassava starch Pinduca	37.8 (±0.03)c
Cassava starch Pinduca + potassium lignate	40.8 (±0.02)bc

(1)Means (±standard error) with different letters are significantly different based on Tukey's studentized range test (P<0.05; n = 4).

Starch composition is determinant in the differences of formulations adherence, and those starches containing amylose and amylopectin produce formulations with high levels of adherence (McGuire & Shasha, 1992). Lignin formulations were reported as improving starch-based formulations (Tamez-Guerra et al., 2000); results from this study (Table 1) did not confirm this previous observation. Differences in starch composition probably affect the interactions with lignin and consequentially adherence.

In general, feeding responses were greater in leaves treated with cucurbitacin-based formulation than in untreated control leaves (Table 2); discrimination occurred in 2.5%, 5%, 10%, and 15% concentrations. The greatest preference index (100) was found at 5% concentration. No differences were detected at 20% concentration treatment and control.

Greater number of insects was found on treated leaves than in control leaves (Table 2); exception occurred in the 20% concentration, in which beetles did not discriminated treated leaves from control ones. These results corroborates previous feeding responses (Table 2), and confirm reports of feeding and arrestment responses to cucurbitacin (Chamblis & Jones, 1966; Howe et al., 1976); hence, on both assessments higher dose was not preferred by beetles; the greatest preference index (93.5) was found at 5% concentration.

Table 2. Feeding and number of *D. speciosa* beetles in paired-choice test, in response to common bean leaves treated or not treated with different doses of cucurbitacin-content formulations in water, in the laboratory(1).

Dose (g 100 mL^{-1})	Treated leaves	Control leaves	Preference index(2)
		Eaten area (cm^2)	
0.25	0.26 (±0.01)a	0.09 (±0.01)b	48.57
0.5	0.41 (±0.02)a	0.00 (±0.00)b	100.00
1.0	0.32 (±0.02)a	0.01 (±0.00)b	93.94
1.5	0.32 (±0.01)a	0.05 (±0.02)b	72.97
2.0	0.13 (±0.01)a	0.10 (±0.01)a	13.04
		Beetles	
0.25	14.20 (±0.93)a	2.70 (±0.35)b	68.05
0.5	11.90 (±0.94)a	0.40 (±0.23)b	93.50
1.0	16.60 (±1.16)a	0.70 (±1.13)b	91.91
1.5	12.80 (±1.05)a	1.50 (±1.15)b	79.02
2.0	6.80 (±0.96)a	4.10 (±1.43)a	24.77

(1)Means (±standard error) followed by the same letter in each line do not differ significantly (P<0.05) using t test. (2)Preference Index = [(T C)/(T+C)]*100, in which T is the eaten area or number of insects feeding on treated leaves; and C is the eaten area or number of insects feeding on untreated control leaves, respectively.

The lack of response to 20% concentration treatment probably reflects the insect behavior in nature; this suggests the existence of a superior threshold of responses.

Spraying cucurbitacin-based formulations affected placement of *D. speciosa* in treated areas (F = 30.96; P>0.00004), 3 and 6 days (F = 73.41; P>0.00001) after spraying. In general, the mean number of insects was higher on treated plots than on control untreated ones (Figure 1). Arresting effect, represented by mean number of insects, was greater in the treatment with the major dosage (3,500 g ha^{-1}), even after a 10 mm rainfall occurrence in the period. Ten days after spraying (total rainfall of 78 mm), populations in treated plots did not differ from untreated controls.

Arrestments of several species of *Diabrotica* to cucurbitacin and their feeding on baits have been previously reported (Metcalf et al., 1987). In the field, the non arrestment after a total 78 mm rainfall suggests that formulations were washed off. However leaf texture probably affect adherence. Further investigations are necessary to establish washoff resistance of formulations on other host plants. In a similar way, studies are desirable to settle alternative spraying configurations, like to target underside of leaves, and hence diminish washoff by rainfalls, as proposed by Chandler & Sutter (1997). Chandler (1998) reported washoff of the aerial applied adherent bait SLAM (Basf Corp., Research Triangle Park, NC and MicroFlo Co., Lakeland, FL) after a short rainfall.

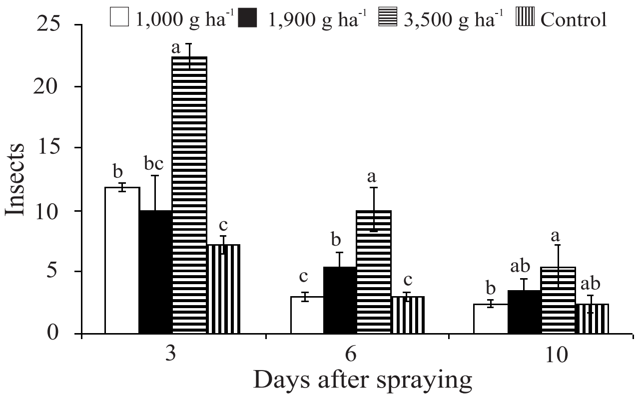

Figure 1. Mean (±standard error) number of *Diabrotica speciosa* beetles in plots treated with cucurbitacin based baits in the field at 3, 6 and 10 days after spraying. Bars with the same letter, at each evaluation, do not differ significantly by Duncan's multiple range test (P<0.05).

Positive responses of *D. speciosa* beetles to treated leaves, in the laboratory and in the field, indicate that cucurbitacin-baited lures could be tested with insecticides. *D. speciosa* insecticides act by contact and ingestion; greater feeding and placement responses related in this work could improve their efficiency. Further investigation concerned with insecticide addition to the lures, and assessments in the field, must be conducted. The relatively great responses of *D. speciosa* to 1,4-dimethoxybenzene (Ventura et al., 2000) suggest this volatile addition to the lures to attract insects, since cucurbitacins are not volatile and do not exhibit long-range attraction.

Common bean is recognized as a very attractive plant to *D. speciosa* (Ventura et al., 1996), and even in the presence of a suitable host plant the insects preferred cucurbitacin-based formulation. Baits are special candidates as suitable tool to *D. speciosa* management in crops in which these insects are root pests.

Conclusions

1. Formulation prepared with cassava starch shows higher adherence than corn starch, and potassium lignate do not improve formulations adherence.
2. Cucurbitacin-content formulation arrests and stimulates feeding of *D. speciosa* beetles.
3. Greatest feeding and arresting preference indices are obtained with 5% cucurbitacin-content concentration in the laboratory.

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