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Thomas J. Weissling
University of Nebraska-Lincoln, tweissling2@unl.edu

Lance J. Meinke
University of Nebraska-Lincoln, lmeinke1@unl.edu

Kandy A. Lytle
University of Nebraska-Lincoln

Effect of Starch-Based Corn Rootworm (Coleoptera: Chrysomelidae) Baits on Selected Nontarget Insect Species: Influence of Semiochemical Composition

THOMAS J. WEISSLING,[1] LANCE J. MEINKE, AND KANDY A. LYTLE

Department of Entomology, University of Nebraska, Lincoln, Nebraska 68583

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ABSTRACT Various starch-encapsulated semiochemical–insecticide formulations, developed for potential use in adult corn rootworm (*Diabrotica* spp.) management programs, were evaluated in the laboratory and field for effectiveness on corn rootworm beetles: a carabid, *Harpalus pennsylvanicus* DeGeer; and a coccinellid, *Coleomegilla maculata lengi* Timberlake. Carbaryl was formulated in pregelatinized starch matrices along with *Diabrotica*-specific semiochemicals. The specific combination of feeding–gustatory stimulants encapsulated within or coating the outside of starch granules significantly influenced effectiveness. All starch formulations containing feeding–gustatory stimulants effectively killed *Diabrotica virgifera virgifera* LeConte adults in laboratory and field bioassays. However, *H. pennsylvanicus* and *C. m. lengi* mortality was greatly reduced when presented with starch granules coated with buffalo gourd (*Cucurbita foetidissima* H.B.K.) root powder (contains cucurbitacin E, I, and E-glycoside) or purified cucurbitacin I. Cucurbitacin I and component(s) of buffalo gourd root powder appear to be *C. m. lengi* and *H. pennsylvanicus* antifeedants. In the field, significantly more *C. m. lengi* and *D. v. virgifera* were collected at traps baited with pollen-coated than root powder-coated starch granules. When granules were broadcast over plants, mortality of *C. m. lengi* was greater in plots receiving pollen-coated than root powder-coated granules whereas the opposite was observed for corn rootworm beetles. Data suggest that to optimize the effectiveness of starch baits against *D. v. virgifera* and to minimize adverse effects on *C. m. lengi* and *H. pennsylvanicus*, granules coated with cucurbitacin rather than with starch or pollen should be used in corn rootworm management programs.

KEY WORDS Insecta, corn rootworms, nontarget species, semiochemicals

BAITS CONTAINING SEMIOCHEMICALS offer several possible advantages for use in pest management systems. Among these is the potential to selectively attract a pest or pest complex to a bait while minimizing adverse effects on nontarget species (Shorey 1981). The corn rootworm species complex (*Diabrotica* spp.) has a well-established array of chemical messengers (Ladd et al. 1983, Lampman & Metcalf 1987, Lampman et al. 1987, Metcalf 1986, Metcalf & Lampman 1989) that make it an ideal system for demonstrating the potential of this concept. *Diabrotica* spp. exhibit species-specific attraction to several related phenylpropanoids (Ladd et al. 1983, Lampman et al. 1987, Metcalf & Lampman 1989) and benezoid compounds (Lampman et al. 1987, Lampman & Metcalf 1987). In addition, cucurbitacins (oxygenated tetracyclic triterpenes) have been identified as compounds that arrest movement and initiate compulsive feeding when detected by diabroticite beetles (Chambliss & Jones 1966, Howe et al. 1976, Metcalf et al. 1980, Metcalf 1986) but tend to repel or deter feeding by non-adapted species (Metcalf et al. 1980, Nielson et al. 1977).

We have used starch matrices, originally developed by Trimnell et al. (1982) and Dunkle & Shasha (1988) as herbicide and *Bacillus thuringiensis* Berliner carriers, respectively, to create controlled release semiochemical–insecticide baits for possible use in adult corn rootworm management programs. Initial studies have shown that *D. virgifera virgifera* LeConte sex pheromone (racemic [Meinke et al. 1989]) and various plant-derived corn rootworm attractants (Weissling et al. 1989) could be successfully encapsulated in starch matrices. Starch matrix formulations have subsequently been developed that contain attractants, feeding–gustatory stimulants (cucurbitacins and pollen), and minute amounts of insecticide, which will attract and kill *D. v. virgifera* over time in field corn (Weissling & Meinke 1991).

Field trials conducted to determine the effect of these starch formulations on corn rootworm beetles and selected nontarget insect species indicated that the carabid *Harpalus pennsylvanicus* DeGeer and the coccinellid *Coleomegilla maculata lengi* Timberlake will feed on starch granules, and that mortality may occur after feeding (Weissling & Meinke

[1] Current address: Fort Lauderdale Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, Fort Lauderdale, Fla. 33314.

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1991). Consequently, laboratory and field studies were conducted to determine if the effectiveness of starch formulations on target and nontarget species could be manipulated by altering the specific combination of feeding-gustatory stimulants (cucurbitacin and pollen) encapsulated within or coating starch granules.

Materials and Methods

Carbaryl and various plant-derived compounds, identified as *Diabrotica* semiochemicals, were used to make starch formulations from pregelatinized starch (Dunkle & Shasha 1988) for use in all experiments. Unless otherwise stated, compounds encapsulated within all test formulations were carbaryl, and the TIC mixture (a volatile corn rootworm attractant composed of a 1:1:1 mixture of 1,2,4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde [Lampman & Metcalf 1987]; each component was purchased from Aldrich Chemical Company, Milwaukee, Wisc.). Carbaryl and the TIC mixture were incorporated into starch matrices at 7.5 and 3 mg/g matrix, respectively. Feeding-gustatory stimulants encapsulated within, or used to coat starch formulations were buffalo gourd (*Cucurbita foetidissima* H.B.K.) root powder (contains cucurbitacins E, I, and E-glycoside [Metcalf et al. 1982]), and pollen (C.C. Pollen Company, Scottsdale, Ariz.). Buffalo gourd root powder from the same source was used in all experiments but cucurbitacin content was not quantified. However, Metcalf et al. (1982) have reported that the amount of cucurbitacin E, I, and E-glycoside in fresh *C. foetidissima* root was 0.28, 1.72, and 0.59 mg/g, respectively. Buffalo gourd root powder and pollen were incorporated into starch matrices at 37.5 and 5 mg/g matrix, respectively (unless otherwise stated). When used to coat starch granules, buffalo gourd root powder and pollen were added to excess.

Laboratory observations have indicated that even when insects are confined within small arenas containing starch-encapsulated semiochemical-insecticide formulations, mortality will occur only after ingestion of granules (T.J.W. & L.J.M., unpublished data). Therefore, all experiments in this study were designed to assay the effect of various feeding-gustatory stimulant combinations placed within or on the outside of starch granules using insecticide induced mortality as an indicator that feeding had occurred.

***Diabrotica v. virgifera* Bioassay.** Laboratory bioassays were used to evaluate the effect of starch formulations on *D. v. virgifera* adults when different feeding-gustatory stimulant combinations were incorporated into or placed on the outside of granules. In the first bioassay, buffalo gourd root powder and pollen were incorporated into the starch formulations at 10 and 2.5 mg/g matrix, respectively. Treatments included granules that were starch-coated, with root powder incorporated into the matrix; starch-coated, with pollen incorporated into the matrix; starch-coated, with root powder and pollen incorporated into the matrix; pollen-coated, with root powder incorporated into the matrix; root powder-coated, with pollen incorporated into the matrix; starch-coated blank granules (no semiochemical or insecticide incorporated into the matrix); and an untreated check (no granules).

Starch granules (0.85–2 mm diameter, 100 mg per treatment per replication) were placed in glass Petri dishes (15 by 60 mm) that were placed in the bottom of cylindrical plastic containers (850 cm^3). Each arena contained a water-moistened cotton wick and was enclosed with a plastic lid that had a circular vent (28 cm^2) covered with nylon mesh. Ten laboratory-reared nondiapause strain *D. v. virgifera* (colony originally obtained from French Agricultural Service, Lamberton, Minn.) were introduced into each arena. Containers were then arranged in a completely random design (three replications) and held at 23 ± 1°C, with a photoperiod of 14:10 (L:D). *D. v. virgifera* mortality was determined at 24 h.

The experiment was repeated with starch formulations that had buffalo gourd root powder and pollen incorporated at 37.5 and 5 mg/g matrix, respectively. Treatments included granules that were starch-coated, with root powder and pollen incorporated into the matrix; pollen-coated, with root powder incorporated into the matrix; root powder-coated, with pollen incorporated into the matrix; starch-coated blank granules (no semiochemicals or insecticide incorporated into the matrix); and an untreated check.

***Coleomegilla maculata lengi* and *H. pennsylvanicus* Bioassay.** The effect of different corn rootworm feeding stimulant combinations was evaluated on two nontarget insect species. *H. pennsylvanicus* and *C. m. lengi* adults were collected in the field and maintained in the laboratory on a diet of *Musca domestica* L. larvae, and various aphid species, respectively, until used in bioassays. Treatments included granules (0.60–0.85 mm diameter) that were starch-coated, with root powder and pollen incorporated into the matrix; root powder-coated, with pollen incorporated into the matrix; pollen-coated, with root powder incorporated into the matrix; and an untreated check.

A separate bioassay was conducted for each nontarget species. Test arenas were plastic Petri dishes (15 by 100 mm) for *H. pennsylvanicus* and 850-cm^3 plastic containers (as described for *D. v. virgifera*) for *C. m. lengi*. Each arena contained a water-moistened cotton wick. Treatments were added to *H. pennsylvanicus* and *C. m. lengi* arenas at 50 and 100 mg per arena, respectively. One *H. pennsylvanicus* or 10 *C. m. lengi* were introduced into appropriate arenas. Test arenas for both species were arranged separately in a completely random design (*H. pennsylvanicus*, 14 replications; *C. m. lengi*, four replications) and were held at 26 ± 1.3°C, with a photoperiod of 14:10 (L:D). Mortality was recorded at 24 h.

Table 1. Mortality of *D. v. virgifera* from ingestion of pregelatinized starch granules formulated with different combinations of feeding-gustatory stimulants

Treatment[a]		% Mortality (24 h), $\bar{x} \pm$ SEM	
Coating	Within	Bioassay 1	Bioassay 2
Starch	BGRP[c]	96.7 ± 3.3a	—
Starch	Pollen[c]	93.3 ± 6.7a	—
Starch	BGRP & Pollen	100.0 ± 0.0a	100.0 ± 0.0a
Pollen	BGRP	100.0 ± 0.0a	96.7 ± 3.3a
BGRP	Pollen	100.0 ± 0.0a	100.0 ± 0.0a
Blank granules		13.3 ± 6.7b	0.0 ± 0.0b
No granules		16.7 ± 3.3b	0.0 ± 0.0b

Means within columns followed by the same letter are not significantly different (least significant difference, $P \leq 0.05$).

[a] Technical carbaryl and TIC (a 1:1:1 mixture of 1,2,4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde) were added to starch formulations at 7.5 mg and 1 mg (each)/g matrix, respectively; coating, outside of matrix; within, encapsulated within matrix.

[b] BGRP: Buffalo gourd (*C. foetidissima* H.B.K.) root powder added to the starch matrix at 10 (bioassay 1) and 37.5 (bioassay 2) mg/g.

[c] Pollen was added to starch formulations at 2.5 (bioassay 1) and 5.0 (bioassay 2) mg/g.

An additional bioassay was done to determine if an alternate food source presented to *C. m. lengi* along with starch formulations influenced mortality. The experimental design was the same as that previously described for *C. m. lengi* except that 57 ± 3 live greenbugs, *Schizaphis graminum* (Rondani), were placed in each arena 30 min before introduction of *C. m. lengi* beetles.

Cucurbita foetidissima root contains several compounds in addition to cucurbitacin (i.e., starch, lignins, and fatty acids [Berry et al. 1978]). Thus, additional bioassays were conducted to determine if purified cucurbitacin would elicit the same responses by nontarget species as observed with buffalo gourd root powder. Arenas used for each nontarget species were identical to those previously described for *H. pennsylvanicus*. Treatments included starch granules (formulated with the TIC-mixture and carbaryl) that were coated with either starch or purified cucurbitacin I (Rotichrom, Atomergic Chemetals Corp., Farmingdale, N.Y.). An untreated check (no granules) was also included. Granules were added to *H. pennsylvanicus* and *C. m. lengi* arenas at 9.9 and 19.8 mg per arena, respectively. One *H. pennsylvanicus* or five *C. m. lengi* were introduced into arenas arranged separately in a completely random design with seven (*H. pennsylvanicus*) and four (*C. m. lengi*) replications. Mortality was recorded at 24 h.

Voucher specimens of *C. m. lengi* and *H. pennsylvanicus* adults were deposited in the University of Nebraska State Museum.

Trapping Study. Starch granules formulated and coated with various feeding-gustatory stimulants were placed in Pherocon 1C traps (Trece, Inc., Salinas, Calif.) from 23 August to 3 September 1989 to evaluate the effect of formulations on corn rootworm beetles and *C. m. lengi* in field corn (*Zea mays* L.). Treatments included granules that were starch-coated, with root powder and pollen incorporated into the matrix; pollen-coated, with root powder incorporated into the matrix; root powder-coated, with pollen incorporated into the matrix; and starch-coated blank granules (no root powder or pollen incorporated into the matrix). Two grams of granules (0.85–2 mm diameter) were placed in plastic Petri dish bottoms (15 by 100 mm) that were taped to the upper surface of adhesive-free trap bottoms. Traps were attached to plants at corn ear height.

Within a 2.25-ha field, treatments were arranged in a randomized complete block design (four replications) and each block (separated by 6.1 m) consisted of four traps spaced 6.1 m apart. All dead or moribund adult *D. v. virgifera*, *D. barberi* Smith & Lawrence, and *C. m. lengi* found in traps or on the soil surface directly beneath traps were collected daily for 10 d. The number of each species collected at each trap was determined, and sex ratios were determined for *D. v. virgifera* and *D. barberi*. Corn ('Pioneer 3377') was in the dough stage at the beginning of the experiment (R4 stage [Ritchie & Hanway 1984]).

To estimate adult corn rootworm and *C. m. lengi* population levels, whole plant beetle counts (Tollefson 1986) were taken on 10 (23 August) and 20 (2 September) randomly selected plants (except those near traps) within the field.

Broadcast Study. Two starch-based formulations coated with different feeding stimulants were compared in a field assay (29 August–3 September 1989) to determine the relative effectiveness of each formulation on adult corn rootworms and two nontarget species. Treatments included starch granules (0.60–4.75 mm diameter) that were root powder-coated, with pollen incorporated into the matrix; and pollen-coated, with root powder incorporated into the matrix. Plots (separated by 3 m) were arranged in a completely random design (four replications) within a 1.13-ha field. Each plot (2.9 m²) included two adjacent corn rows. Treatments were applied to plots on 28 August by sprinkling 4 g (13 kg/ha) of granules by hand evenly over plants in both rows. Plants at the time of application had just completed flowering ('Pioneer 3377' R2 stage [Ritchie & Hanway 1984]). Efficacy of treatments was determined daily by collecting all dead or moribund adult *D. v. virgifera*, *D. barberi*, *D. undecimpunctata howardi* Barber, *H. pennsylvanicus*, and *C. m. lengi* within plots on plants and on the soil surface.

To estimate adult corn rootworm and *C. m. lengi* populations in the test field, whole plant beetle counts were taken on 15 randomly selected plants (excluding those within plots) on 29 August and 3 September.

Statistical Analyses. All data (except in tests where purified cucurbitacin I was used) were subjected to angular [arcsine $\sqrt{x}$ (laboratory studies)] and square root $(x + 0.5)$ (field studies) transfor-

Table 2. Mortality ($\bar{x}$ ± SEM) of *C. m. lengi* and *H. pennsylvanicus* from ingestion of pregelatinized starch granules formulated with different combinations of feeding–gustatory stimulants

Treatment[a]		% Mortality (24 h), $\bar{x}$ ± SEM		
Coating	Within	*H. pennsylvanicus*	*C. m. lengi*	*C. m. lengi*[b]
Starch	BGRP[c] & Pollen[d]	100.0 ± 0.0a	100.0 ± 0.0a	97.5 ± 2.5a
Pollen	BGRP	71.4 ± 12.5b	97.5 ± 2.5a	100.0 ± 0.0a
BGRP	Pollen	35.7 ± 13.3c	47.5 ± 1.0b	15.0 ± 6.5b
No granules		0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c

Means within columns followed by the same letter are not significantly different (least significant difference, $P \leq 0.05$).
[a] Technical carbaryl and TIC (a 1:1:1 mixture of 1,2,4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde) were added to starch formulations at 7.5 mg and 1 mg (each)/g matrix, respectively; coating, outside of matrix; within, encapsulated within matrix.
[b] An average of 57 live greenbugs (*S. graminum*) were added to each arena just before introduction of *C. m. lengi*.
[c] BGRP: Buffalo gourd (*C. foetidissima* H.B.K.) root powder added to starch formulations at 37.5 mg/g.
[d] Pollen was added to starch formulations at 5.0 mg/g.

mation and were analyzed by analysis of variance (ANOVA) (SAS Institute 1985). Untransformed means are presented in tables. Analyses were conducted for treatment effects over the entire experimental period (two-way ANOVA [laboratory studies] and split-plot design [field studies]). Least significant difference tests (SAS Institute 1985) were used for mean separation where significant ($P \leq 0.05$) treatment effects occurred. Mortality data in tests where purified cucurbitacin I was used were ranked and analyzed by the Kruskal–Wallis test (χ^2 approximation) (SAS Institute 1985).

Results

***Diabrotica v. virgifera* Bioassay.** *D. v. virgifera* mortality attributable to ingestion of starch formulations ranged from 93 to 100% after 24 h (Table 1). Statistical analyses indicated significant treatment effects for both bioassays (bioassay 1: $F = 92.93$; df = 6,12; $P < 0.01$, bioassay 2: $F = 1{,}321.0$; df = 4,8; $P < 0.01$). *D. v. virgifera* mortality in both bioassays was significantly greater in treatments with insecticide incorporated into the formulations than in treatments with blank granules or in the untreated check. There were no significant differences in *D. v. virgifera* mortality among formulations that contained insecticide (Table 1).

***Coleomegilla maculata lengi* and *H. pennsylvanicus* Bioassay.** *H. pennsylvanicus* mortality varied significantly among treatments ($F = 26.79$; df = 3,39; $P < 0.01$). *H. pennsylvanicus* mortality in arenas containing starch formulations was significantly greater than in the untreated check (Table 2). However, mortality was significantly greater in arenas with starch- and pollen-coated formulations than in arenas with the root powder-coated formulation. Significantly greater *H. pennsylvanicus* mortality was observed in arenas that contained starch-coated granules than in arenas with pollen-coated granules (Table 2).

Coleomegilla maculata lengi mortality also differed significantly among treatments (without aphids: $F = 75.42$; df = 3,9; $P < 0.01$, with aphids: $F = 249.4$; df = 3,9; $P < 0.01$) (Table 2). In both bioassays, *C. m. lengi* mortality in arenas with starch granules was significantly greater than in arenas without granules. In addition, *C. m. lengi* mortality in arenas containing starch- or pollen-coated granules was significantly greater than in arenas with root powder-coated granules (Table 2).

Analyses of *C. m. lengi* and *H. pennsylvanicus* mortality in bioassays where cucurbitacin I-coated granules were used indicated that significant differences occurred among treatments (*C. m. lengi*: $\chi^2 = 10.67$; df = 2; $P = 0.005$, *H. pennsylvanicus*: $\chi^2 = 9.41$; df = 2; $P = 0.009$). *C. m. lengi* and *H. pennsylvanicus* mortality in arenas containing starch-coated granules was significantly greater than mortality in the other treatments (percentage mortality [$\bar{x}$ ± SEM] starch-coated granules: *C. m. lengi*; 95.0 ± 5.0, *H. pennsylvanicus*; 57.1 ± 20.0). No mortality was observed in arenas containing cucurbitacin I-coated granules or in the untreated check at 24 h.

Trapping Study. Total rainfall received during the experimental period was 0.8 cm on 2 September (measured 200 m from the study site). No *C. m. lengi* beetles were detected during whole-plant counts and the *D. v. virgifera* and *D. barberi* population levels declined during the experimental period (beetles per plant [$\bar{x}$ ± SEM]: *D. v. virgifera*: 23 August, 0.80 ± 0.20; 2 September, 0.0 ± 0.0; *D. barberi*: 23 August, 0.10 ± 0.10; 2 September, 0.0 ± 0.0).

Analysis of trap catch over the entire experimental period indicated that statistical differences occurred among treatments (*D. v. virgifera*: $F = 13.06$; df = 3,9; $P < 0.01$, *D. barberi*: $F = 88.61$; df = 3,9; $P < 0.01$, *C. m. lengi*: $F = 14.57$; df = 3,9; $P < 0.01$), and among dates (*D. v. virgifera*: $F = 27.55$; df = 9,81; $P < 0.01$, *D. barberi*: $F = 8.93$; df = 9,81; $P < 0.01$, *C. m. lengi*: $F = 2.25$; df = 9,81; $P < 0.03$). Significant treatment × date interactions were also observed (*D. v. virgifera*: $F = 5.23$; df = 27,81; $P < 0.01$, *D. barberi*: $F = 3.79$; df = 27,81; $P < 0.01$).

Significantly more *D. v. virgifera* were collected at traps baited with pollen-coated starch granules than at traps baited with any other treatment whereas significantly more *D. barberi* were collected at traps baited with starch-coated granules

Table 3. Mean number ± SEM of *D. v. virgifera*, *D. barberi*, and *C. m. lengi* collected per trap per day for 10 d at traps baited with pregelatinized starch granules formulated with different combinations of feeding–gustatory stimulants, 1989

Treatment[a]		*D. v. virgifera*		*D. barberi*		*C. m. lengi*
Coating	Within	No.	♀:♂[b]	No.	♀:♂	No.
Starch	BGRP[c] & Pollen[d]	5.8 ± 0.8b	4.2	2.4 ± 0.5a	0.02	0.1 ± 0.1b
Pollen	BGRP	14.2 ± 1.7a	26.3	0.8 ± 0.2c	0.03	0.3 ± 0.1a
BGRP	Pollen	8.9 ± 1.2b	10.5	1.4 ± 0.3b	0.14	0.0 ± 0.0b
Starch	—	0.2 ± 0.1c	5.0	0.0 ± 0.0d	—	0.0 ± 0.0b

Means within columns followed by the same letter are not significantly different (least significant difference, $P \leq 0.05$).

[a] Technical carbaryl and TIC (a 1:1:1 mixture of 1,2,4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde) were added to starch formulations at 7.5 mg and 1 mg (each)/g matrix, respectively; coating, outside of matrix; within, encapsulated within matrix.

[b] ♀:♂, mean number of female corn rootworms per mean number of male corn rootworms collected from traps.

[c] BGRP: Buffalo gourd (*C. foetidissima* H.B.K.) root powder added to starch formulations at 37.5 mg/g.

[d] Pollen was added to starch formulations at 5.0 mg/g.

than at traps baited with any other treatment (Table 3). The mean number of *D. v. virgifera* collected at traps baited with starch- and root powder-coated granules was similar and significantly more beetles were collected at these traps than at traps baited with blank granules. The mean number of *D. barberi* collected at traps baited with root powder-coated granules was significantly greater than the mean number collected at traps baited with pollen-coated granules. In addition, significantly more *D. barberi* were collected at traps baited with pollen-coated granules than at traps baited with blank granules (Table 3). Significantly more *C. m. lengi* were collected at traps baited with pollen-coated granules than at traps baited with any other treatment (Table 3).

The *D. v. virgifera* female-to-male ratio varied among treatments but females were always more numerous than males. In all cases, more male *D. barberi* were caught in traps than females (Table 3).

Broadcast Study. Total rainfall received during the experimental period was 0.8 cm on 2 September (measured 300 m from the study site). *D. v. virgifera*, *D. barberi*, *D. u. howardi*, and *C. m. lengi* population levels decreased during the experimental period (number per plant [$\bar{x} \pm$ SEM]: *D. v. virgifera*: 29 August, 2.40 ± 0.58, 3 September, 1.13 ± 0.39; *D. barberi*: 29 August, 0.93 ± 0.28, 3 September, 0.33 ± 0.13; *D. u. howardi*: 29 August, 0.07 ± 0.07, 3 September, 0.00 ± 0.00; *C. m. lengi*: 29 August, 0.33 ± 0.13, 3 September, 0.07 ± 0.07).

The analysis conducted on mortality of each species over the entire experimental period indicated that statistical differences occurred among treatments (*D. u. howardi*: $F = 26.98$; df = 1,3; $P < 0.01$, *C. m. lengi*: $F = 11.66$; df = 1,3; $P < 0.04$), and among dates (*D. v. virgifera*: $F = 8.90$; df = 4,12; $P < 0.01$, *D. barberi*: $F = 18.56$; df = 4,12; $P < 0.01$, *C. m. lengi*: $F = 13.48$; df = 4,12; $P < 0.01$). Significant treatment × date interactions were also observed (*D. barberi*: $F = 4.86$; df = 4,12; $P < 0.01$, *C. m. lengi*: $F = 9.62$; df = 4,12; $P < 0.01$). *H. pennsylvanicus* were collected from the treated plots but levels were low and therefore were not included in the analyses.

Significantly fewer *C. m. lengi* beetles were collected from plots treated with root powder-coated granules than plots treated with pollen-coated granules (Table 4). In all cases, more corn rootworm beetles were collected from plots treated with root powder-coated granules, than from plots that received pollen-coated granules, but differences were significant only for *D. u. howardi* (Table 4). Female-to-male ratios, determined from collected dead corn rootworm beetles, were skewed towards females for *D. v. virgifera*, and males for *D. barberi* and *D. u. howardi*.

Discussion

All starch formulations evaluated were effective as *D. v. virgifera* mortality agents when feeding–gustatory stimulants were present either within or coating the granules. However, the specific combination of feeding–gustatory stimulants appears to be an important factor in determining whether nontarget species accept or reject starch granules as a potential food source.

Diabrotica v. virgifera mortalities in laboratory bioassays and in the broadcast study were similar among starch granule treatments with insecticide. However, *D. v. virgifera* mortality varied among treatments in the trapping study. The trapping study was conducted during the postflowering phenology stage when corn rootworm populations were low and decreased through time. The amount of preferred food (i.e., fresh or green silks and pollen, Lance & Fisher 1987, Naranjo & Sawyer 1987) available to corn rootworm beetles is limited during this period, and *D. v. virgifera* beetles may have perceived pollen-coated granules placed in traps as a potential food source. Reasons why *D. barberi* preferred starch-coated granules over other granules are unclear. The broadcast study was conducted in a late-planted field where scattered flowering corn plants (sources of quality food) were still present and corn rootworm populations were relatively high. Pollen-coated granules placed in this

Table 4. Mean number ± SEM of dead or moribund *D. v. virgifera*, *D. barberi*, *D. u. howardi*, and *C. m. lengi* collected per plot per day for 5 d from plots that received broadcast applied pregelatinized starch granules formulated with different feeding–gustatory stimulants, 1989

Treatment[a]		*D. v. virgifera*		*D. barberi*		*D. u. howardi*		*C. m. lengi*
Coating	Within	No.	♀:♂[b]	No.	♀:♂	No.	♀:♂	No.
Pollen	BGRP[c]	9.5 ± 2.1a	2.2	1.2 ± 0.4a	0.9	0.5 ± 0.1b	0.1	2.7 ± 0.7a
BGRP	Pollen[d]	22.5 ± 5.4a	2.6	3.5 ± 1.0a	0.6	3.5 ± 0.5a	0.1	0.1 ± 0.1b

Means within columns followed by the same letter are not significantly different (least significant difference, $P \leq 0.05$).

[a] Technical carbaryl and TIC (a 1:1:1 mixture of 1,2,4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde) were added to starch formulations at 7.5 mg and 1 mg (each)/g matrix, respectively; coating, outside of matrix; within, encapsulated within matrix.

[b] ♀:♂, mean number of female corn rootworms/mean number of male corn rootworms collected from treated plots.

[c] BGRP: Buffalo gourd (*C. foetidissima* H.B.K.) root powder added to starch formulations at 37.5 mg/g.

[d] Pollen was added to the starch formulations at 5.0 mg/g.

field may not have been as apparent to corn rootworm beetles as a potential food source as granules in the trapping study because of possible competition from flowering corn plants. Decreasing beetle populations and variability in the amount of food present in the fields may have contributed to the significant interactions observed through time in both experiments.

Mortality of *H. pennsylvanicus* and *C. m. lengi* in laboratory bioassays was greatly reduced in treatments where buffalo gourd root powder or cucurbitacin I was used to coat granules. This suggests that even in a no-choice situation, where hunger could potentially reduce diet choice selectivity over time (Dethier 1982), cucurbitacin I and a component of buffalo gourd root powder (presumably cucurbitacin E, I, or E-glycoside) are adult *C. m. lengi* and *H. pennsylvanicus* antifeedants as defined by Frazier (1986). Cucurbitacins are known feeding stimulants for diabroticites (Chambliss & Jones 1966) but also deter feeding by such insects as the chrysomelid beetles *Phyllotreta nemorum* L. (Nielson et al. 1977) and *Cerotoma trifurcata* (Forster) (Metcalf et al. 1980) when applied to host plant tissues.

Results from field studies suggest that when given a choice, *C. m. lengi* will feed more readily on pollen-coated than starch- or buffalo gourd root powder-coated starch formulations. Pollen is an accepted food of most coccinellids (Hodek 1967, Conrad 1959) and like *D. v. virgifera*, *C. m. lengi* may have perceived pollen-coated granules as a potential food source. Pollen could have been especially apparent to *C. m. lengi* during the trapping study because of the advanced corn phenology and the absence of alternate food sources (i.e., aphids) in the field (T.J.W., unpublished data).

Alternate food sources available to nontarget species could potentially decrease starch formulation induced mortality by reducing hunger and subsequent encounters with granules. When released into arenas with aphids, *C. m. lengi* beetles randomly searched the enclosures and ingested acceptable food sources as they were encountered (L.J.M. & K.A.L., unpublished data). *C. m. lengi* mortality in arenas containing aphids and buffalo gourd root powder-coated granules was substantially lower than mortality in the bioassay where root powder-coated granules were presented to *C. m. lengi* without the addition of aphids (Table 2). This suggests that the presence of an alternate food source may have increased the likelihood of *C. m. lengi* beetles rejecting root powder-coated granules as a potential food source. *C. m. lengi* mortality at 24 h in other treatments appeared to be unaffected by the addition of aphids into arenas (Table 2).

Maintenance of nontarget species (especially beneficial species) populations after implementation of control tactics may be of great importance, especially when outbreaks of secondary pests are a concern. Data presented here indicate that starch granules formulated with different feeding–gustatory stimulants have potential for manipulating the feeding preference of nontarget species while maintaining efficacy against a target pest. Specifically, to optimize the effectiveness of starch baits against *D. v. virgifera* and to minimize adverse effects on *C. m. lengi* or *H. pennsylvanicus*, starch-based granules coated with cucurbitacin would be preferred over starch- or pollen-coated granules for use in corn rootworm management programs.

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