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# Toxicity of insecticide-bait mixtures to insecticide resistant and susceptible western corn rootworms (Coleoptera: Chrysomelidae)

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## Abstract

Organophosphate resistant and susceptible populations of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. were subjected to adult feeding bioassays with different combinations of insecticide and a cucurbitacin bait. Five technical grade insecticides (methyl-parathion, carbaryl, fipronil, bifenthrin and indoxacarb) were used in combination with Invite EC™ as the feeding stimulant. Differences in susceptibility to the insecticide/bait combinations were observed among the resistant and susceptible populations for methyl-parathion and carbaryl. Susceptibility to fipronil, bifenthrin and indoxacarb was similar among the resistant and susceptible populations. Assays in which response to the insecticide/bait combination was compared with the bait alone indicated that methyl-parathion and bifenthrin were deterrent when compared to other treatments. These results suggest that the efficacy of Invite as a feeding stimulant in combination with certain insecticides may be compromised by previously identified resistance and by insecticides that antagonize the feeding stimulation of the cucurbitacin bait.

*Keywords:* Western corn rootworm; Insecticide baits; Invite EC; Carbaryl; Methyl-parathion; Resistance

## 1. Introduction

Traditional management of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in the United States Corn Belt involves either the application of a soil insecticide to control larval root feeding, crop rotation with a non-host crop, or adult management with aerial applications of insecticides targeted to reduce oviposition (Pruess et al., 1974; Mayo and Peters, 1978; Branson and Krysan, 1981; Levine and Oloumi-Sadeghi, 1991). In certain regions of Nebraska, long-term use of contact organophosphate and carbamate insecticides for adult control has resulted in significant levels of resistance to microencapsulated methyl-parathion (Penncap-M) and carbaryl among populations of western corn rootworms (Meinke et al., 1998; Siegfried et al., 1998; Wright et al., 2000). A new strain of the western corn rootworm has recently been identified in certain portions of Illinois and Indiana with a propensity to feed and oviposit in soybean fields (Levine and Gray, 1996) thus circumventing crop rotation as a management strategy.

The development of insecticide resistance and behavioral adaptation to crop rotation has renewed interest in using semiochemical (cucurbitacin)-based baits containing reduced doses of insecticides in adult management programs (Weissling et al., 1991; Lance and Sutter, 1992; Brust and Foster, 1995; Chandler et al., 1995; Schroder et al., 1998). Cucurbitacins, a class of secondary plant compounds common to the members of family Cucurbitaceae, stimulate compulsive feeding and arrestant responses in the diabroticite beetles (Metcalf and Lampman, 1989; Tallamy and Halaweish, 1993; DeMilo et al., 1998). The USDA-ARS Areawide

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Management Program for corn rootworms (Chandler, 1998) focuses on adult management with the cucurbitacin-based insecticide baits that reduce the rate of use and environmental contamination relative to soil insecticides and other adulticides.

There are three commercially available products in the market for beetle control that use the semiochemical-based technology. These include an insecticide bait, SLAM® (buffalo gourd [*Cucurbita foetidissima* H.B.K.], root powder, 87%, with 13% carbaryl) (Micro Flo Inc., Memphis, TN), and two adjuvant-based products, CideTrak CRW® (Trece Inc., Salinas, CA), and Invite EC™ (Florida Food Products Agrochemicals, Eustis, FL). CideTrak is a wettable powder containing buffalo gourd root powder and Invite is an emulsifiable concentrate containing Hawkesbury watermelon, *Citrullus lanatus* (Thunb.) Matsum & Nakai (Syn. *Citrullus vulgaris* Schrad), juice. SLAM® has been shown to be effective in controlling corn rootworm populations in different corn growing states of the mid-western US (Chandler, 1998; Tollefson, 1998; Wilde et al., 1998; Pingel et al., 2001). Recent studies by Schroder et al. (1998, 2001) demonstrated that Invite (water-soluble formulation containing cucurbitacin), was effective in controlling rootworm adults when applied in combination with a toxic photoactive xanthene dye, Phloxine B: D & C Red Dye No.28.

During 2001–2002, Invite EC has been commercially available and recommended for use with insecticide formulations containing methyl-parathion and carbaryl as active ingredients throughout the Corn Belt (http://www.floridafood.com/invite.html) including Nebraska, where resistance to these compounds has been previously reported (Meinke et al., 1998; Scharf et al., 1999). The objectives of the present study were to compare the susceptibility of previously identified insecticide-resistant and susceptible western corn rootworm populations to different insecticides used in combination with Invite and determine the compatibility of a variety of active ingredient insecticides with the Invite formulation.

## 2. Materials and methods

### 2.1. Insect populations

Insecticide resistant (York and Phelps Counties) and susceptible (Saunders County) western corn rootworm populations from Nebraska were collected in 1997 and maintained for four generations in laboratory colonies at the USDA-ARS Northern Grain Insects Research Laboratory in Brookings, South Dakota. Susceptibility of these populations to methyl-parathion and carbaryl was previously characterized with both topical application and residual exposure to treated glass vials (Meinke et al., 1998; Miota et al., 1998; Scharf et al., 1999, 2000). Adult rootworms were shipped from the rearing facility when the sex ratio of emergent beetles approached 1:1 and within 48 h after emergence. Adult rootworms were maintained in Plexiglas cages at 22–25°C on fresh sweet corn ears and lettuce. Diabroticite beetle sensitivity to cucurbitacins is influenced by age, sex and reproductive activity (Tallamy and Halaweish, 1993). Care was taken to avoid exposure of beetles to diet containing cucurbitacins, and the beetles were of similar age (7–10 days old) when used in bioassays and feeding behavior assays.

### 2.2. Chemicals

Technical grade carbaryl (99.5% AI), methyl-parathion (99% AI) and bifenthrin (99% AI) were purchased from Chem Services Inc. (West Chester, PA). Fipronil (97.1% AI) was provided by Rhône Poulenc Ag Co. (Research Triangle Park, NC). Indoxacarb (79% AI) was provided by E. I. DuPont Co. (Wilmington, DE). All insecticide dilutions were prepared in reagent grade acetone ($>99.5\%$ purity; EM Science, Gibbstown, NJ). Invite EC™ (80% principal functioning agent) was obtained from Florida Food Products Agrochemicals (Eustis, FL).

### 2.3. Bioassays

Bioassays were performed with the five technical insecticides described above, using a range of concentrations in combination with a single Invite concentration. A series of insecticide concentrations (methyl-parathion and carbaryl: 1.5625–100 mg/ml, fipronil: 0.625–20 mg/ml, bifenthrin: 2.5–40 mg/ml and indoxacarb: 3.125–100 mg/ml) in acetone were diluted 10-fold with Invite, which was previously diluted 10-fold with water. Five 20 μl droplets of each insecticide/Invite dilution were uniformly distributed onto a Whatman No. 2 filter paper (Maidstone, England) in a disposable plastic Petri dish (100 × 15 mm). Ten beetles were held in each dish in total darkness at 22°C, and mortality was determined after 24 h as the lack of coordinated beetle movement. Each insecticide concentration was replicated 5 times for each population.

### 2.4. Behavioral assays

No choice feeding behavior assays were conducted with adult rootworms from the susceptible Saunders County population. Seven treatments were evaluated, consisting of Invite alone, water, and five insecticide/Invite combinations (methyl-parathion, carbaryl, fipronil, bifenthrin and indoxacarb) at the rootworm $LC_{50}$ for each insecticide as determined previously for the susceptible population. Each treatment was dispensed onto a regenerated cellulose membrane disk (0.45 μm pore size, 13 mm$^2$ diameter, Schleicher and Schuell, Keene, NH) in a 20 μl droplet (Hollister and Mullin, 1999). Air-dried disks were positioned on minuten pins above moistened Whatman No. 2 filter paper in Petri dishes containing a paraffin bottom. Each treatment was replicated 10 times with four beetles (two each of male and female) placed in each Petri dish and held at 22°C in total darkness. The number of beetles resting or feeding on the treated disk was recorded at 15 and 30 min after introduction. The consumed disk areas were measured after 6 h of feeding to the nearest 0.01 cm$^2$ using a LICOR-3000 leaf area meter (LICOR, Lincoln, NE). Mortality of beetles exposed to treated discs was less than 5% at 1 hr for all insecticide-bait combinations. Ten disks were used to calculate the mean disk area before feeding. Percent disk consumption was calculated by subtracting the area consumed from the mean disk area.

### 2.5. Statistical analysis

Lethal concentration data were analyzed by probit analysis (Finney, 1971) using POLO-PC (LeOra Software, 1987). The method of Robertson and Priesler (1992) was used to calculate confidence intervals for resistance ratios. With this test, if the 95% confidence interval calculated for a ratio does not include 1.0, a significant difference exists between the values being compared.

The percent disk consumption data were transformed by arcsine $(P)^{1/2}$ prior to analysis and differences in percent disk consumption by beetles between treatments were analyzed with PROC ANOVA and means were compared using the Tukey's HSD (Highest Significant Difference) test at $\alpha = 0.05$ (SAS Institute, 2001). PROC MIXED was used to analyze the beetle count data and treatment means were compared using linear contrasts.

## 3. Results

### 3.1. Insecticide bioassays

Results of insecticide bioassays to estimate susceptibility of the three populations to five insecticides in combination with the Invite formulation are presented in Table 1. Resistant populations used in this study demonstrated statistically higher $LC_{50}$ values when compared to the susceptible Saunders County population for both methyl-parathion and carbaryl (Table 1). However, these resistant populations were not

Table 1
Probit analysis of mortality of insecticide resistant and susceptible populations of *D.v. virgifera* exposed to various insecticides combined with Invite EC

| Insecticide[a] | Population | $n$[b] | $\chi$[c] | Slope (±SE) | $LC_{50}$ (95% CI)[d] | $RR_{50}$[e] (95% CI) | $LC_{90}$ (95% CI)[d] | $RR_{90}$[e] (95% CI) |
|---|---|---|---|---|---|---|---|---|
| Methyl-parathion | Saunders Co. (S) | 300 | 15.22* | 5.0 (0.57) | 0.62 (0.42–0.94) | — | 1.12 (0.78–3.32) | — |
| | York Co. (R) | 300 | 0.37 | 1.8 (0.25) | 6.79 (5.25–9.71) | 11.0 (6.09–19.6) | 34.5 (20.4–84.3) | 30.8 (10.5–88.9) |
| | Phelps Co. (R) | 300 | 0.87 | 1.6 (0.27) | 12.6 (8.50–25.6) | 20.4 (10.5–41.2) | 84.4 (37.0–440.5) | 77.2 (17.8–333) |
| Carbaryl | Saunders Co. (S) | 350 | 4.48* | 3.4 (0.36) | 0.42 (0.33–0.53) | — | 1.01 (0.76–1.60) | — |
| | York Co. (R) | 300 | 0.68 | 3.1 (0.38) | 1.12 (1.31–2.34) | 2.66 (1.84–3.90) | 2.87 (2.34–3.85) | 2.84 (1.57–2.23) |
| | Phelps Co. (R) | 300 | 1.96 | 3.2 (0.37) | 1.23 (1.04–1.44) | 2.94 (1.88–4.62) | 3.14 (2.57–4.18) | 3.11 (1.61–6.06) |
| Fipronil | Saunders Co. (S) | 300 | 7.33* | 3.2 (0.35) | 0.15 (0.092–0.23) | — | 0.38 (0.24–1.28) | — |
| | York Co. (R) | 250 | 1.82 | 3.3 (0.41) | 0.23 (0.20–0.27) | 1.57 (0.99–2.31) | 0.57 (0.47–0.78) | 1.51 (0.79–2.77) |
| | Phelps Co. (R) | 250 | 0.97 | 3.8 (0.49) | 0.22 (0.19–0.25) | 1.47 (1.00–2.17) | 0.47 (0.40–0.62) | 1.24 (0.68–2.29) |
| Bifenthrin | Saunders Co. (S) | 300 | 2.90 | 2.7 (0.28) | 0.83 (0.70–0.98) | — | 2.52 (1.99–3.52) | — |
| | York Co. (R) | 250 | 3.83* | 3.4 (0.40) | 1.21 (0.64–2.19) | 1.46 (1.00–2.05) | 2.89 (1.98–6.80) | 1.15 (0.61–2.06) |
| | Phelps Co. (R) | 250 | 2.93* | 3.2 (0.38) | 1.27 (0.75–2.07) | 1.52 (0.97–2.40) | 3.16 (1.97–15.0) | 1.25 (0.61–2.58) |
| Indoxacarb | Saunders Co. (S) | 350 | 2.01 | 2.6 (0.24) | 1.54 (1.30–1.82) | — | 4.79 (3.80–6.53) | — |
| | York Co. (R) | 350 | 3.65 | 3.1 (0.30) | 2.25 (1.93–2.62) | 1.46 (1.01–2.11) | 5.81 (4.71–7.70) | 1.21 (0.67–2.21) |
| | Phelps Co. (R) | 350 | 5.25* | 2.8 (0.27) | 2.25 (1.73–2.96) | 1.46 (0.98–2.20) | 6.41 (4.56–11.2) | 1.34 (0.71–2.54) |

[a] All insecticide dilutions in acetone were mixed with Invite EC in a 1:9 ratio. Invite used in the mixtures was 10-fold diluted with water.

[b] Number of insects used in each probit analysis.

[c] Chi-square goodness-of-fit as determined using POLO-PC and departures from an expected model based on heterogeneity factor >1.0 are represented by an '*'.

[d] Lethal concentrations (in mg/ml) with 95% confidence intervals (CI) at the 50% ($LC_{50}$) and 90% ($LC_{90}$) levels of probit mortality.

[e] Resistance ratios indicate the fold-difference when compared to susceptible Saunders Co. population at $LC_{50}$ and $LC_{90}$. If the 95% confidence interval calculated for a ratio does not include 1.0, a significant difference exists.

significantly different from the susceptible population in their susceptibility to fipronil, indoxacarb and bifenthrin at both the $LC_{50}$ and $LC_{90}$ (Table 1). These results are consistent with previously reported resistance levels for exposure to surface residues (Miota et al., 1998; Scharf et al., 1999, 2000) and topical bioassays (Meinke et al., 1998; Parimi, unpublished data).

### 3.2. Feeding behavior assays

There were significant differences among treatments in the number of beetles observed feeding and/or resting on the treated disk at 15 ($P<0.01$) and 30 min ($P=0.01$) (Fig. 1A and B). The number of beetles found feeding and/or resting on treated disks was significantly greater for Invite alone (15 and 30 min) and Invite + carbaryl (15 min), when compared with other treatments. Bifenthrin, methyl-parathion and indoxacarb appeared to deter the adult rootworms away from the treated disks throughout the experiment. The response to indoxacarb in combination with Invite was significantly greater than the water-treated disks at 15 min, although significantly less than the Invite formulation alone.

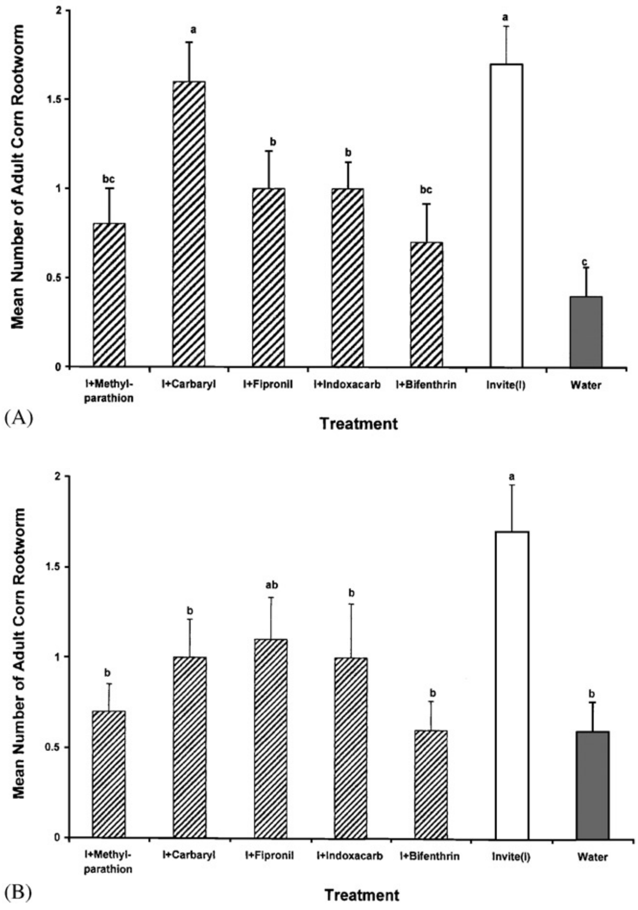

Fig. 1. Mean number of susceptible *D.v. virgifera* beetles observed feeding and/or resting on disks treated with Invite alone, Invite + insecticide (bifenthrin, carbaryl, fipronil, indoxacarb and methyl-parathion), or water alone, in a no choice behavior assay at two time periods ((A) 15 min and (B) 30 min) after the beetles were introduced. Means followed by the same letter are not significantly different (Linear Contrasts, $P>0.05$).

The analysis of disk consumption demonstrated significant differences among treatments ($P<0.01$) (Fig. 2). All disks treated with Invite + insecticide had significantly less area consumed relative to disks treated with Invite alone. However, disks treated with carbaryl, fipronil and indoxacarb with Invite, exhibited significantly greater consumption relative to the water-treated control disks. Consumption of disks treated with methyl-parathion + Invite or bifenthrin + Invite were not significantly different from disk treated with water.

## 4. Discussion

The results of this investigation provide insight into the potential use of Invite as bait in combination with different insecticides for adult corn rootworm management. Bioassays of insecticide/Invite combinations indicated that both York and Phelps populations are resistant to methyl-parathion, and to some extent carbaryl, when the insecticide was ingested in combination with Invite. The levels of resistance are consistent with those reported previously for both topically applied insecticide and residual exposure to insecticide-treated glass surfaces (Meinke et al., 1998; Miota et al., 1998; Scharf et al., 1999, 2000; Parimi, unpublished data), although somewhat lower resistance levels were observed for carbaryl in combination with Invite compared to other exposure routes. Detoxification enzymes, such as cytochrome P450 and hydrolytic enzymes are concentrated in gut tissues of western corn rootworm (Siegfried and Mullin, 1988; Siegfried, unpublished), and both enzyme systems have been shown to be involved in resistance to carbaryl and methyl-parathion (Miota et al., 1998; Scharf et al., 1999, 2000). Consequently, it is not unexpected that resistance to ingested insecticide is similar to the levels reported for other exposure routes.

The feeding behavior assays indicate that not all active ingredients are equally compatible with the Invite formulation because some compounds appeared to deter initial response and consumption of the cucurbitacin baits. The inhibition in response and feeding seemed to increase with time of exposure especially for carbaryl, and could be related to initial neurotoxicity associated with exposure to the insecticides. It should be noted that although significant inhibition of beetle response to and consumption of treated discs was observed for all the Invite/insecticide combinations, significant response curves were obtained for all five compounds

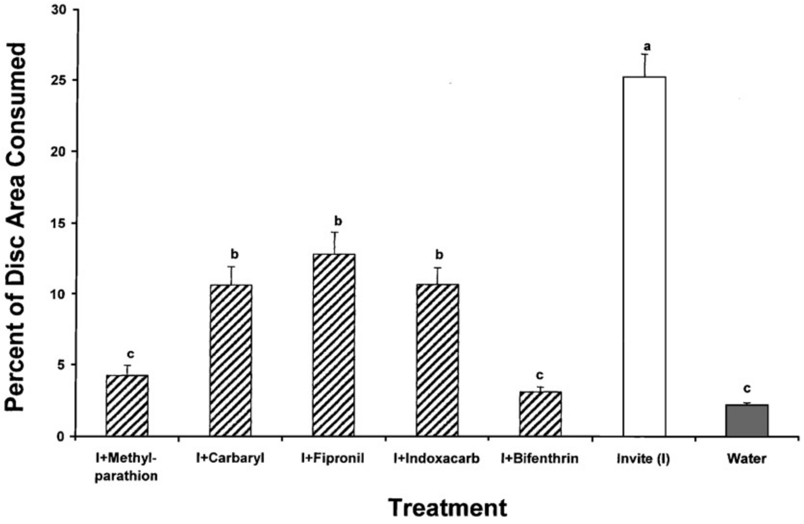

Fig. 2. Percent mean consumption of cellulose membrane disks treated with Invite alone, Invite + insecticide (bifenthrin, carbaryl, fipronil, indoxacarb and methyl-parathion) baits, or water alone, by susceptible *D.v. virgifera* beetles, in a no choice feeding behavior assay. Means followed by the same letter are not significantly different (Tukey's HSD test, $P > 0.05$). Data was transformed by arcsine $(P)^{1/2}$ for analysis; however, actual numerical values are presented in the figure.

when the Invite formulation was combined with increasing concentrations of insecticide in 24 h exposures (Table 1). These results indicate that the beetles did consume some of the toxin and were not completely inhibited from responding to the bait. However, these data may not be indicative of a field response where beetles could respond by movement away from a treated field as opposed to confinement in close proximity to the insecticide/bait combination.

Zhu et al. (2001) indicated reduced carbaryl susceptibility of western corn rootworm populations from areas treated repeatedly with SLAM (cucurbitacin-based formulation of carbaryl) after a relatively short period (4 yr) of use for rootworm management. Additionally, our results suggest that once resistance is present in a population, cucurbitacin-based methyl-parathion or carbaryl baits may be less effective for adult rootworm management. One strategy suggested for insecticide resistance management is to rotate insecticides of different modes of action with cucurbitacin-based baits (Siegfried et al., 1998). Both indoxacarb and fipronil could provide alternatives to carbaryl as both represent unique modes of action, susceptibility to both was unaffected by resistance and, both were comparable in terms of feeding response to the cucurbitacin bait.

Overall, the feeding behavior of the susceptible population and tolerance differences observed among different populations and insecticide bait mixtures, suggest that this technology should be used with caution in different corn-growing areas of the United States, especially in the regions where organophosphate resistance has been previously documented.

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