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Toxicity of a formulation of the insecticide indoxacarb to the tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae), and the big-eyed bug, *Geocoris punctipes* (Hemiptera: Lygaeidae)

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Abstract: Indoxacarb is a new oxadiazine insecticide that has shown outstanding field insecticidal activity. The toxicity of a 145 g litre^{-1} indoxacarb SC formulation (Steward®) was studied on the tarnished plant bug *Lygus lineolaris* and the big-eyed bug *Geocoris punctipes*. Both insect species responded very similarly to indoxacarb in topical, tarsal contact and plant feeding toxicity studies. The topical LD_{50} of the formulation was *c* 35 ng AI per insect for both species. Prolonged tarsal contact with dry indoxacarb residues did not result in mortality for either insect species. However, both species were susceptible to feeding through dried residues of indoxacarb after spraying on young cotton plants. Feeding on water-washed plants resulted in lower mortality than that observed with unwashed plants, and toxicity declined even more dramatically after a detergent rinse, indicating that much of the indoxacarb probably resides on the cotton leaf surface or in the waxy cuticle. These results were corroborated by HPLC–mass spectrometry measurements of indoxacarb residues on the plants. Greater mortality for both species was observed in a higher relative humidity environment. Higher levels of accumulated indoxacarb and its active metabolite were detected in dead *G punctipes* than in *L lineolaris* after feeding on sprayed, unwashed plants. When female *G punctipes* ate indoxacarb-treated *Heliothis zea* eggs, there was significant toxicity. However, only *c* 15% of the females consumed indoxacarb-treated eggs, and the rest of the females showed a significant diminution of feeding in response to the insecticide. Cotton field studies have shown that indoxacarb treatments at labelled rates lead to a dramatic decline in *L lineolaris*, with negligible declines in beneficial populations. A major route of intoxication of *L lineolaris* in indoxacarb-treated cotton fields thus appears to be via oral, and not cuticular, uptake of residues from treated cotton plants. The mechanisms for selectivity/safety for *G punctipes* are currently under investigation and may be a combination of differential feeding behavior and diminution of feeding by females exposed to indoxacarb-treated eggs.
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Keywords: Steward®; indoxacarb; insecticide susceptibility; *Lygus lineolaris*; *Geocoris punctipes*

1 INTRODUCTION

Indoxacarb (DPX-MP062) is a new oxadiazine insecticide, discovered by the EI DuPont Co, which has shown outstanding field insecticidal activity, environmental compatibility and safety to non-target organisms.[1,2] Indoxacarb is especially active on foliar-feeding lepidopteran larvae; the oral toxicity has been found to be 0.01 and 0.03 ng mg^{-1} body weight for *Heliothis virescens* (F) and *Spodoptera frugiperda* (Smith), respectively.[2] Metabolism studies in several lepidopteran larvae have shown that orally administered indoxacarb is rapidly bioactivated to its sodium channel blocking *N*-decarbomethoxylated metabolite (DCMP).[2,3] Bioactivation occurred more slowly after topical treatment.[2] When lepidopteran larvae ingest sprayed foliage, or are sprayed directly, they stop feeding and either go into mild convulsions or a passive paralysis from which there is no recovery.[3] One of the formulations of indoxacarb is a 145 g litre^{-1} suspension concentrate with the trade name Steward® in the USA. This material has shown considerable promise for cotton insect control in the USA.[4,5]

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is a pest in cotton in the south-eastern USA. [6]*L lineolaris* may become a critical pest in this region with the commercialization

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of *Bt* cotton and the successful eradication of the boll weevil. Development of insecticide-resistant populations is of major concern because of the growing focus on this pest as a target for insecticide treatment and the paucity of alternative insecticides.[7] The big-eyed bug, *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae), is a predator of many pest species, including *H virescens* and *Helicoverpa zea* (Boddie) eggs and small larvae.[8] *Geocoris punctipes* also feeds on plants, increasing the likelihood of its survival during the absence of invertebrate hosts.[9] Indoxacarb is highly efficacious against *L lineolaris* and may be a new tool in an integrated pest management program for this pest in cotton.[10] Since the pest and natural enemy species are both sucking insects and can occur in cotton fields concurrently, selectivity of indoxacarb with respect to these two insect species is an important issue in an integrated pest management program. Results from small field tests have shown that populations of *L lineolaris* are dramatically suppressed by applications of indoxacarb.[10] However, *G punctipes* were not affected adversely by applications of indoxacarb in small field-plot tests.[11] The specific objectives of the present study were (1) to determine critical mechanisms of intoxication of *L lineolaris* after indoxacarb treatment and (2) to determine possible explanations for the selectivity to indoxacarb observed between *L lineolaris* and *G punctipes*. Thus, we bioassayed both insects via topical, tarsal contact and oral (sprayed plants) routes of administration, using HPLC–MS measurements of indoxacarb levels in cotton plants and insects where appropriate, to understand the differential susceptibility of these two insects. We also examined the toxicological consequences of rinsing off the indoxacarb residues from plants and, finally, the susceptibility of *G punctipes* to indoxacarb-treated lepidopteran eggs.

2 MATERIALS AND METHODS

2.1 Insects

Lygus lineolaris and *G punctipes* adult females were collected using a sweep net from flowering wild mustard in Tifton, GA. Only fresh, newly-collected and active *L lineolaris* and *G punctipes* females were used for tests. Eggs of *H zea* were obtained from a colony reared in the USDA, ARS rearing laboratory at Tifton, GA on an agar soybean flour-wheat germ diet at 26 °C, 50 (±5)% RH, and a 15:9 h light:dark photoperiod.[12]

2.2 Chemicals

Indoxacarb (DPX-MP062) 145 g litre^{-1} suspension concentrate (Steward®) was provided by DuPont Agricultural Products, Wilmington, DE. HPLC-grade water and acetonitrile were from EM Science (Gibbstown, NJ). Collagenase and chitinase for extraction of insect tissues were obtained from Sigma (St Louis, MO).

2.3 Topical bioassays

Indoxacarb SC was diluted with water to provide the required range of doses. All concentrations are given in terms of AI. A group of five adult insects in Petri dishes (100 mm × 15 mm) were anesthetized with carbon dioxide for *c* 45 s and a 0.2-µl droplet of insecticide or water control was placed on the dorsal thorax of each anesthetized insect using a microapplicator (Hamilton PB600 Dispenser; Hamilton, Reno, NV). Each treated insect was fed (potatoes for *L lineolaris* and *H zea* eggs for *G punctipes*) and held in Petri dishes (100 mm × 15 mm) under ambient laboratory conditions (22 (±2) °C, 50–60% RH). Mortality was determined 24, 48 and 72 h after treatment. Experiments were replicated from six to fourteen times. Dead insects were collected at the end of the test. Three *L lineolaris* adults and five *G punctipes* adults were placed in vials (approximately 20 mg total insect weight per vial) and weighted. The vials were then frozen and prepared for indoxacarb analysis by HPLC–MS. Topical toxicity data were analyzed by the SAS PROBIT procedure (SAS Institute, Cary, NC). LD_{50} values were considered significantly different if the 95% confidence limits did not overlap. Concentrations of indoxacarb and DCMP in dead insects were analyzed using *t*-tests.

2.4 Tarsal contact bioassays

Serial dilutions of indoxacarb SC were prepared using water as the diluent. A cotton leaf (first fully expanded leaf in terminal; variety DP 5415) was collected from the plant and placed in a clean plastic Petri dish (100 mm × 15 mm). A Potter spray tower (Burkard Manufacturing Co Ltd, Hertfordshire, UK) with an air pressure of 1.47×10^5 Pa was used to spray cotton leaves. After treated leaves were placed individually in clean Petri dishes, five insects were placed on the treated leaf. Insects walked on treated leaves for 4 h without feeding (by active intervention) and then were removed to clean Petri dishes without cotton leaves. All treated insects were fed (potatoes for *L lineolaris* and *H zea* eggs for *G punctipes*) and held under ambient laboratory conditions (22 (±2) °C, 50–60% RH). Mortality was determined 48 h after treatment. Experiments were replicated eight times. Residual toxicity data were analyzed by the SAS PROBIT procedure (SAS Institute, Cary, NC). LC_{50} values were considered significantly different if the 95% confidence limits did not overlap.

2.5 Plant feeding bioassays

2.5.1 General procedures

Cotton (variety DP 5415) plants were grown and fertilized (Osmocote®) in pots in the greenhouse at 25–30 °C and 50–70% RH, with a 16:8 h light:dark cycle. Plants were 7–9 weeks old when used in experiments. Plants, except for controls, were sprayed with indoxacarb SC at a rate of 0.1 kg AI ha^{-1} using a two-row spray boom calibrated to deliver 112.25 litre ha^{-1} using a TX-8 nozzle (Spraying Systems, Wheaton,

IL) while maintaining an air pressure of 2.75×10^5 Pa and a speed of 1.86 km h^{-1}. The final concentration was thus 890 μg indoxacarb ml^{-1} spray suspension. The height of the nozzle above the plant was *c* 30 cm. Four treatment replicates were sprayed separately. The plants were allowed to dry for a minimum of 2 h.

The terminal (top 15 cm) of the plant was then clipped off. Terminals were cut 0 (2 h), 3 and 5 days after the insecticide application. Some clipped terminals were washed before insect exposure. For the water wash, the clipped terminal was washed by swirling in water (2 × 20 ml) for 30 s using a 50-ml disposable polypropylene centrifuge tube (Corning Inc, Corning, NY). For the detergent wash, the clipped terminal was washed in an aqueous dilution of liquid detergent (Dawn®, 100 ml $litre^{-1}$; 2 × 500 ml) for 1 min. After the washing procedure, stems of both washed and unwashed clipped terminals were placed individually in cups of wet sand to keep the terminals fresh. Clipped terminals in sand-cups were placed individually in a cardboard carton or plastic cylinder. The carton was a 3.8-litre ice cream container with organdy covering the top of the cage. The plastic cylinder (31.8 cm high; 9.2 cm diameter) was covered tightly with a plastic cup. When plant cages were established, 20 insects of either species were placed in each cage. Mortality for each species was recorded 24, 48 and 72 h after treatment.

2.5.2 Test 1: effect of water washes on toxicity of sprayed cotton plants

The three treatments included unwashed, water-washed and control terminals in plastic cylinder plant cages. Dead insects were collected at the end of the test. Three *L lineolaris* adults or five *G punctipes* adults were placed in vials (*c* 20 mg total insect weight per vial) and weighed. Vials were held on dry ice before preparation for HPLC–MS indoxacarb analysis. After the experiment, the top (*c* 5 cm) of the cotton terminal was clipped and weighed. The plant tissue was washed by swirling in water (2 × 10 ml) for 15 s in a 50-ml centrifuge tube. All washed plant tissues were placed in water in a separate tube and held on dry ice before preparation for HPLC–MS analysis. Percentage mortality data were compared between species for the same compound using *t*-tests and between wash regimes using the SAS ANOVA procedure (SAS Institute, Cary, NC) followed by least significant difference (LSD) separation of means. The amount of indoxacarb in treated cotton terminals was compared between wash regimes by the SAS ANOVA procedure followed by LSD separation of means. The concentrations of indoxacarb and DCMP in dead insects were analyzed using *t*-tests.

2.5.3 Test 2: effect of relative humidity on toxicity of sprayed cotton plants

The two treatments were unwashed and control terminals; the unwashed terminals were placed in both carton (*c* 52% RH) and plastic cylinder (*c* 86% RH) plant cages, while controls were in a cardboard plant cages. Temperature and relative humidity readings from the inside of the plant cages were obtained using a HI 8564 thermohygrometer (Hanna Instruments, Inc, Woonsocket, RI). Relative humidity and percentage mortality data were compared between treatments by the SAS ANOVA procedure followed by LSD separation of means. Percentage mortality data were compared between species using *t*-tests.

2.5.4 Test 3: effect of detergent washes on toxicity of sprayed cotton plants

The three treatments comprised unwashed, detergent-washed and control terminals in plastic cylinder cages; only *L lineolaris* were tested. Percentage mortality data were analyzed by the SAS ANOVA procedure followed by LSD separation of means.

2.6 Extraction/processing insect samples for HPLC–MS

Intoxicated insects from selected bioassay experiments were weighed, rinsed with acetone (2 × 5 ml) to remove surface residues, pooled to achieve *c* 20 mg insect tissue per sample, homogenized in microfuge tubes with a handheld Dremel motor and Kontes plastic pestle (5000 rev min^{-1}), and then incubated with a mixture of chitinase (5 mg ml^{-1}, Sigma C-6137) and collagenase (10 mg ml^{-1}, Sigma C-9891) for 24 h at 23 °C. A twofold volume of acetonitrile was then added, the sample chilled to 4 °C and centrifuged at 15 800 rpm for 5 min. The supernatant was removed, placed into another microfuge tube, chilled to −20 °C, brought back to 23 °C, chilled again to 4 °C and centrifuged as previously. The supernatant was diluted threefold with HPLC-grade acetonitrile for direct analysis by HPLC-electrospray MS. Experiments to monitor recoveries from extraction processes using ^{14}C-labelled compound indicate that >90% of total radiocarbon administered is recovered as oxadiazines over the time course of these experiments.

2.7 Extraction/processing cotton leaves for HPLC–MS

The leaves were dried in a hood to remove moisture while pooled in the 50-ml centrifuge tubes. Leaves were weighed and then bead milled using two 0.5-cm steel ball bearings in a 2-ml conical screwcap microfuge tube (Bio Plas, San Francisco, CA) with a Mini-Bead Beater (BioSpec Products, Bartlesville, OK) at 4200 rev min^{-1} for 60 s. An acetonitrile rinse (1 ml) of the sample tubes was added to the bead-mill microfuge tubes, and the samples were bead milled again. The bead milled microfuge tubes were chilled to 4 °C, centrifuged (15 800 *g*, 5 min, 4 °C), and 1 ml of the supernatant removed for electrospray HPLC–MS as described in Section 2.8.

2.8 HPLC–MS conditions

Insect and plant samples were analyzed for indoxacarb

and DCMP by electrospray HPLC–MS. HPLC separation was performed on a 4.6 mm × 50 mm, 5 μm packing, Zorbax® Stable-Bond™ C18 column using a Hewlett-Packard HP1100 HPLC with binary pumping system and column oven set to 40 °C (Agilent Technologies, Wilmington, DE). The mobile phase was an acetonitrile–water gradient, each with 0.1% formic acid (25–90% acetonitrile over 3 min.), delivered at 1.0 ml min^{-1}. The liquid flow was split post-column approximately 1:10, with approximately 100 μl min^{-1} flow introduced to the mass spectrometer. A Micromass Quattro II triple-stage quadrupole mass spectrometer (Beverly, MA) equipped with an electrospray source was used. The mass spectrometer was operated in positive ion mode, with a spray voltage of 3.2 kV and a source temperature of 100 °C. Indoxacarb and its *N*-decarbomethoxylated metabolite (DCMP) were monitored in multiple reaction monitoring (MRM) mode using transitions of 528–>218 and 470–>267, respectively. Collision energies were 25 and 15 V, respectively, with argon gas introduced into the collision cell so as to increase the pressure 1×10^3 Pa.

2.9 *Geocoris punctipes* treated-egg bioassay

This bioassay used only *G punctipes* females that had shown an ability to consume at least five *H zea* eggs over an 8-h period. After eating eggs, these females were starved overnight and then aspirated singly into experimental Petri dishes (100 mm × 15 mm). The three experimental treatments were: (1) 20 *H zea* eggs (treated) on filter paper treated with indoxacarb SC at 0.1 kg AI ha^{-1} (0.09 lb AI $acre^{-1}$), (2) 20 untreated *H zea* eggs on filter paper treated with indoxacarb SC at 0.1 kg AI ha^{-1}, and (3) untreated control with 20 untreated *H zea* eggs on untreated filter paper. To avoid egg hatch during the test, *H zea* eggs were irradiated with 230 Gy at 20 °C with a well-type Co^{60} source (Gammarad irradiator, Model GR-12, US Nuclear Division, Irvine, CA) at the rate of 23 Gy min^{-1} (±5%, X-ray monitor probe calibration). *Geocoris punctipes* can only eat *H zea* eggs that are anchored to some surface. Unfortunately, testing treated eggs with untreated filter paper could not be accomplished since removing treated eggs from any surface contaminated the eggs; eggs were therefore placed on very small (2.3 cm) filter paper (Whatman International Ltd, Maidstone, England) to limit *G punctipes* exposure to indoxacarb on filter paper, and an indoxacarb-treated filter paper control was included in the test. Treatments were sprayed using a Potter spray tower (Burkard Manufacturing Co Ltd, Hertfordshire, UK) with an air pressure of 1.47×10^5 Pa. After spraying, treated filter paper with or without eggs was placed in a clean Petri dish free of indoxacarb residues. Filter paper and eggs were allowed to dry for 1 h before aspirating females individually into Petri dishes (100 mm × 15 mm). Twenty-five females were used for each replicate for the filter paper control, while 30 females per replicate were used for the other two treatments. The test was replicated four times. All treatment dishes were held in a rearing room maintained at *c* 27 °C and 50–60% RH. The percentages of females that consumed eggs were determined 24, 48, 72 and 96 h after treatment (HAT) and compared by ANOVA followed by LSD separation of means. New unsprayed eggs (20 *H zea* per Petri dish) and filter paper were given to females at each of these time periods. Data on female mortality were determined at 96 HAT and compared between females that consumed and did not consume eggs using *t*-tests and among egg treatments by the ANOVA procedure followed by LSD separation of means.

3 RESULTS AND DISCUSSION

3.1 Topical bioassays

The topical susceptibilities of *L lineolaris* and *G punctipes* to indoxacarb were very similar (Table 1), the LD_{50} for both insects being approximately 35 ng indoxacarb per insect. *Geocoris punctipes* accumulated more indoxacarb and converted more to DCMP (Table 2) but, since the LD_{50} values were the same, this insect apparently can tolerate higher body concentrations of indoxacarb than *L lineolaris*.

3.2 Tarsal contact bioassays

Neither *L lineolaris* nor *G punctipes* were sensitive to indoxacarb after walking on dried residues on cotton plants, with LC_{50} values of >3000 μg indoxacarb ml^{-1} (Table 3). Apparently tarsal contact is an inefficient mode of uptake. Residue studies conducted in scintillation vials resulted in similar findings (Snodgrass, GL, pers comm, 2000).

Table 1. Topical toxicity of indoxacarb to adult *Lygus lineolaris* and *Geocoris punctipes* 24, 48 and 72 h after treatment (HAT)

HAT	Species	n	Slope (±SE)	LD_{50} (ng per insect) (95% CI)[a]
24	*G punctipes*	510	1.70 (±0.16)	97 (79–121)
	L lineolaris	445	0.50 (±0.19)	>240
48	*G punctipes*	510	1.65 (±0.14)	47 (38–58)
	L lineolaris	445	1.29 (±0.15)	84 (65–118)
72	*G punctipes*	510	1.63 (±0.14)	33 (26–40)
	L lineolaris	445	1.54 (±0.15)	37 (30–47)

[a] Control mortality ranges were 0–5.0% and 1.7–6.7% for *G punctipes* and *L lineolaris*, respectively. Average body weights were 6.5 mg for *L lineolaris* and 4.0 mg for *G punctipes*.

Table 2. Concentration of indoxacarb and DCMP in dead *Geocoris punctipes* and *Lygus lineolaris* after topical treatment with indoxacarb

Concentration applied ($\mu g\ ml^{-1}$)	Indoxacarb ($ng\ mg^{-1}$ body wt) (±SE)[a]				DCMP ($ng\ mg^{-1}$ body wt) (±SE)[a]	
	G punctipes	n	L lineolaris	n	G punctipes	L lineolaris
1200	5.30 (±1.2) a,1	9	0.52 (±0.1) b,1	11	0.39 (±0.1) a,2	0.15 (±0) b,2
600	2.05 (±0.9) a,1	4	0.16 (±0) b,1	8	0.25 (±0.1) a,2	0.06 (±0) b,2
300	0.43 (±0.1) a,1	3	0.02 (±0) b,1	8	0.06 (±0) a,2	0.002 (±0) b,2
150	0.19 (±0) a,1	2	0.01 (±0) b,1	3	0.07 (±0) a,2	0 b,2

[a] Means within a row followed by the same letter are not significantly different ($P > 0.05$; *t*-test) between species for a single compound. Means within a row followed by the same number are not significantly different ($P > 0.05$; *t*-test) between compounds for a single species.

Table 3. Toxicity of indoxacarb-treated cotton leaves to adult *Lygus lineolaris* and *Geocoris punctipes* by tarsal contact 48h after treatment

Species	n	Slope (SE)	LC_{50} (μg AI ml^{-1}) (95% CI)[a]
G punctipes	200	1.86 (0.36)	3782 (2813–>4800)
L lineolaris	200	2.94 (0.52)	3999 (3250–>4800)

[a] Control mortality was 10.0% and 0% for *G punctipes* and *L lineolaris*, respectively.

3.3 Plant feeding bioassays

3.3.1 Test 1: effect of water washes on toxicity of sprayed cotton plants

The mortalities observed for both species after feeding through dried indoxacarb residues on cotton terminals were similar for all treatments (Table 4). Unwashed terminals led to approximately 90% mortality at day 0, and 50% mortality at 5 days for both species. The insecticide rates used in this test compare well with field-use rates of indoxacarb SC; the sprayed rate of 0.1 kg AI ha^{-1} at 112.25 litre ha^{-1} is approximately 890 μg AI ml^{-1} and helps to explain the excellent potency to *L lineolaris* observed with indoxacarb-treated cotton fields. Previous laboratory bioassays have shown that indoxacarb was active against *L lineolaris* that fed on this insecticide on an inert matrix.[10] The water-washed cotton terminals still retained insecticidal activity, although at a significantly lower level than in unwashed plants. The relative difference in insecticidal activity between unwashed and water-washed plants was more pronounced at 5 days than at 3 days application. The activity in washed plants is probably due to indoxacarb that has penetrated the cuticle and is refractory to water washout. HPLC–MS results certainly showed indoxacarb present in washed leaves (0.57 (± 0.47) $\mu g\ g^{-1}$ plant weight), although in approximately fourfold lower concentrations than in unwashed leaves (2.26 (± 1.05) $\mu g\ g^{-1}$ plant weight). Note that this experiment measured total indoxacarb on and in the leaf, without discerning the detailed microlocalization of the compound.

More indoxacarb was observed in dead *L lineolaris* than in *G punctipes* when they fed on unwashed plants rather than on washed plants. *L lineolaris* fed more on the plant and had accumulated more indoxacarb than *G punctipes*, but the amount of DCMP in dead insects on washed plants was the same (Table 5). Very few insects were dead in the wash treatment at 5 days after treatment, but unwashed plants still showed excellent insecticidal activity, which is an indication of the residuality of indoxacarb in cotton fields.

Table 4. The effect of different wash regimens on toxicity of indoxacarb-treated cotton terminals to *Lygus lineolaris* and *Geocoris punctipes* 0 (2h), 3 and 5d after treatment (DAT)

DAT	Wash treatment[a]	Mortality (%) (±SE)[b]	
		G punctipes	L lineolaris
0	Unwashed	90.9 (±5.2) a,1	96.1 (±3.5) a,1
	Water-washed	35.6 (±8.7) a,2	45.8 (±10.0) a,2
	Control	4.0 (±2.3) a,3	4.0 (±2.1) a,3
3	Unwashed	80.0 (±5.8) a,1	75.0 (±7.4) a,1
	Water-washed	10.3 (±4.0) a,2	12.6 (±3.2) a,2
	Control	2.8 (±1.3) a,2	4.1 (±2.4) a,2
5	Unwashed	50.9 (±17.9) a,1	52.6 (±11.8) a,1
	Water-washed	1.9 (±1.4) 2a,2	3.7 (±3.2) a,2
	Control	5.0 (±0) a,2	3.7 (±3.2) a,2

[a] $n = 4$.
[b] Mortality was assessed 72h after treatment. Means within a row followed by the same letter are not significantly different ($P > 0.05$; *t*-test) between species for a single treatment for a single day. Means within a column followed by the same number are not significantly different ($P > 0.05$; LSD) between treatments for a single species for a single day.

3.3.2 Test 2: effect of relative humidity on toxicity of sprayed cotton plants

Higher mortality occurred for each species in the plant cages with the higher relative humidity than in the low humidity environment (Table 6). There was no statistical difference ($F = 0.28$; $df = 44$; $P = 0.7594$) in temperature between the plant cages: mean cylinder temperature was 22.43 (±0.19) °C, mean carton temperature was 22.34 (±0.19) °C and mean control temperature was 22.26 (±0.13) °C. Mortality progressively increased over 72h after treatment (Table 7) indicating that, while potent, indoxacarb is not a rapid knockdown agent against these species. These sucking insects are thus capable of absorbing and bioactivating indoxacarb after oral administration, but they do so more slowly than the Lepidoptera.[2]

Table 5. Concentration of indoxacarb and DCMP in dead *Geocoris punctipes* and *Lygus lineolaris* after feeding on indoxacarb-treated cotton terminals 0 (2h), 3, and 5 days after treatment (DAT)

		Indoxacarb (ng mg^{-1} body wt) (±SE)[a]				*DCMP (ng mg^{-1} body wt) (±SE)*[a]	
DAT	*Treatment*	G punctipes	n	L lineolaris	n	G punctipes	L lineolaris
0	Unwashed	0.19 (±0.03) A,1,a	17	0.63 (±0.09) A,1,b	28	0.12 (±0.02) B,1,a	0.25 (±0.03) B,1,b
	Water-washed	0.27 (±0.04) A,1,a	8	0.42 (±0.14) A,1,a	10	0.14 (±0.02) B,1,a	0.16 (±0.04) B,1,a
3	Unwashed	0.23 (±0.03) A,1,a	13	0.38 (±0.03) A,1,b	22	0.14 (±0.03) B,1,a	0.28 (±0.03) B,1,b
	Water-washed	0.07 (±0.08) A,1,a	2	0.02 (±0.11) A,2,a	3	0.15 (±0.10) A,1,a	0.10 (±0.08) A,2,a
5	Unwashed	0.13 (±0.03) A,a	9	0.24 (±0.03) A,b	18	0.07 (±0.01) B,a	0.13 (±0.02) B,b

[a] Means within a row followed by the same capital letter are not significantly different ($P>0.05$; *t*-test) between compounds for a single species. Means within a column followed by the same number are not significantly different ($P>0.05$; *t*-test) between treatments for a single day. Means within a row followed by the same lower case letter are not significantly different ($P>0.05$; *t*-test) between species for a single compound.

Table 6. The effect of relative humidity on toxicity of indoxacarb-treated cotton terminals to *Lygus lineolaris* and *Geocoris punctipes* 0 (2h), 3 and 5 days after treatment (DAT)

			Mortality (%) (±SE)[c]	
DAT	*Treatment*[a]	*RH (%) (±SE)*[b]	L lineolaris	G punctipes
0	Cylinder	86.2 (±1.1) A	83.5 (±6.8) a,1	75.8 (±5.3) a,1
	Carton	56.6 (±1.4) B	26.6 (±2.4) a,2	44.0 (±6.0) b,2
	Control	53.9 (±0.9) B	1.6 (±1.1) a,3	2.9 (±2.4) a,3
3	Cylinder	84.7 (±1.7) A	73.2 (±8.3) a,1	63.8 (±9.5) a,1
	Carton	54.3 (±1.5) B	27.0 (±2.6) a,2	24.8 (±5.3) a,2
	Control	51.2 (±1.1) B	5.2 (±1.9) a,3	1.6 (±1.1) a,3
5	Cylinder	87.9 (±1.5) A	53.8 (±3.2) a,1	57.2 (±11.4) a,1
	Carton	48.0 (±0.4) B	26.4 (±2.7) a,2	4.0 (±2.3) b,2
	Control	48.6 (±1.2) B	7.8 (±2.4) a,3	2.8 (±1.3) a,2

[a] $n=4$.
[b] Means within a column followed by the same capital letter are not significantly different ($P>0.05$; LSD) between treatments for a single day.
[c] Mortality was assessed 72h after treatment. Means within a row followed by the same lowercase letter are not significantly different ($P>0.05$; *t*-test) between species for a single treatment for a single day. Means within a column followed by the same number are not significantly different ($P>0.05$; LSD) between treatments for a single day.

3.3.3 *Test 3: effect of detergent wash on toxicity of sprayed cotton plants*

Exposure to the unwashed cotton terminals resulted in the highest mortality for *L lineolaris* (Table 8). Mean temperature and relative humidity for all plant cages was 22.38 (±1.28) °C and 89.4 (±0.5) % RH, respectively. Washing the cotton terminals with detergent eliminated the insecticidal activity of the plants. This wash may have removed the cuticle from the plant, and it appears that little indoxacarb remained in the leaf

Table 7. Toxicity of unwashed indoxacarb-treated cotton terminals in cylinder cages to *Lygus lineolaris* and *Geocoris punctipes* 24, 48 and 72h after treatment (HAT)

	Mortality (%) (±SE)[b]	
HAT[a]	L lineolaris	G punctipes
24	12.6 (±4.3) a,1	12.2 (±8.7) a,1
48	31.7 (±15.3) a,1	26.7 (±9.5) a,1
72	83.5 (±6.8) a,2	75.8 (±5.3) a,2

[a] $n=4$.
[b] Means within a row followed by the same letter are not significantly different ($P>0.05$; *t*-test) between species for a single treatment. Means within a column followed by the same number are not significantly different ($P>0.05$; LSD) between treatments. Control mortality ranges were 0–2.5% and 0–1.3% for *G punctipes* and *L lineolaris*, respectively.

Table 8. Effect of different wash regimens on toxicity of indoxacarb-treated cotton terminals to *Lygus lineolaris* 0 (2h), 3 and 5 days after treatment (DAT)

DAT	*Wash treatment*[a]	*Mortality (%) (±SE)*[b]
0	Unwashed	77.9 (±2.1) a
	Detergent-washed	7.4 (±4.0) b
	Control	5.5 (±2.9) b
3	Unwashed	72.2 (±4.2) a
	Detergent-washed	5.2 (±1.9) b
	Control	9.2 (±2.7) b
5	Unwashed	36.7 (±5.4) a
	Detergent-washed	15.0 (±2.0) b
	Control	8.2 (±3.5) b

[a] $n=4$.
[b] Mortality was assessed 72h after treatment. Means followed by the same letter are not significantly different ($P>0.05$; LSD) between wash treatments for a single day.

cells in a form which was bioavailable to the insects. The water wash data demonstrated that some indoxacarb was present in the cuticle. However, since *L lineolaris* mortality was higher when the leaves were not washed than when they were washed with either water or detergent, more indoxacarb must be present on the surface of the leaves than in the cuticle or cotton leaf cells when indoxacarb is sprayed on cotton terminals.

3.4 *Geocoris punctipes* treated-egg bioassay

Indoxacarb significantly reduced the percentage of *G punctipes* females that consumed eggs 24 HAT (Table 9), indicating that either *G punctipes* females were repelled by the insecticide or that it acted as a feeding inhibitor. Even after treated eggs were removed 24 HAT, females previously exposed to indoxacarb-treated eggs were less likely to consume eggs than females given untreated eggs, suggesting that indoxacarb was acting as a feeding inhibitor, not killing females but somehow lowering selected females' ability to feed. By 96 HAT, most of the live females in all treatments were eating eggs, and so feeding inhibition appeared to be reversible after 96h.

Geocoris punctipes feeds by inserting the proboscis into an egg and sucking the inside of the egg into the digestive tract of the insect. The observed reduction in feeding could be attributed to the toxicity of DCMP in *H zea* eggs. Treated eggs of *H zea* are likely to have accumulated indoxacarb and converted it to DCMP rapidly throughout the course of the experiment. Therefore, it is likely that active metabolite may have intoxicated *G punctipes* by affecting the ion channels of cells surrounding the midgut with a subsequent slow recovery. Indoxacarb was probably ingested when the proboscis encountered residues of the insecticide on the filter paper. In the field, *G punctipes* normally also feeds on cotton plants, and so it was not surprising when we observed these females probing indoxacarb-treated filter paper during the test. Since indoxacarb has no tarsal contact activity for this insect species, any effect in the filter paper treatment must be attributable to oral uptake of the insecticide while probing. Females probably ingested more indoxacarb when consuming indoxacarb-treated eggs than when probing filter paper with dried residues of indoxacarb since a greater reduction in females consuming eggs occurred in egg-treated dishes than in only filter paper-treated dishes.

To understand better the effect of indoxacarb-treated eggs on *G punctipes* females, the mortality of females that had consumed eggs (consumers) *versus* those that had not consumed eggs (non-consumers) was examined 96 HAT (Table 10). For consumers and non-consumers, mortality was highest for treated eggs, lower for untreated eggs on treated filter paper and non-existent for the untreated control. Even though the non-consumers that died had not eaten any eggs, they probably had ingested some indoxacarb by probing the surface of a chorion or filter paper treated with the compound. Mortality was similar for consumers and non-consumers for the filter paper treatment, so mortality was due only to probing treated filter paper. Mortality was much higher for consumers than non-consumers for the treated eggs, suggesting that females that ate eggs ingested more indoxacarb than non-consumers. Since only about 15% of the females in the test were consumers, total mortality for all females was only 41.7% when exposed to indoxacarb-treated eggs. Thus, inhibition of feeding by the females in indoxacarb-treated dishes observed earlier in the test actually reduced overall mortality for these females. Some of the mortality that occurred in indoxacarb-treated egg dishes could be attributed to probing treated filter, and thus mortality due only to consuming treated eggs was probably less than could be assessed or *c* 20%. Even so, the mortality of *G punctipes* females was moderately low when these females were given eggs treated with indoxacarb.

HAT[a]	*Egg treatment*[b]	*Females consuming eggs (%) (±SE)*[c]
24	Treated[d] eggs on treated filter paper	15.0 (±3.0) a
	Untreated eggs on treated filter paper	40.1 (±5.9) b
	Untreated control	66.4 (±4.0) c
48	Treated eggs on treated filter paper	48.3 (±3.1) a
	Untreated eggs on treated filter paper	47.0 (±3.8) a
	Untreated control	64.8 (±5.4) b
72	Treated eggs on treated filter paper	69.7 (±3.8) a
	Untreated eggs on treated filter paper	82.9 (±1.3) b
	Untreated control	91.6 (±2.1) c
96	Treated eggs on treated filter paper	87.4 (±2.3) a
	Untreated eggs on treated filter paper	88.2 (±2.3) a
	Untreated control	91.5 (±3.3) a

[a] Treated eggs and filter paper removed 24 HAT.
[b] $n = 4$.
[c] Means within a column followed by the same lower case letter are not significantly different ($P > 0.05$; LSD) among treatments for a single HAT category.
[d] Treated with indoxacarb at 0.01 kg AI ha^{-1}.

Table 9. *Geocoris punctipes* females consuming eggs 24, 48, 72 and 96h after treatment (HAT) for three egg treatments

Table 10. Mortality 96 h after treatment (HAT) for *Geocoris punctipes* females which had consumed eggs (consumers) and those which had not consumed eggs (non-consumers) 24 h after being exposed to three egg treatments

	Consumers at 24 HAT	*Non-consumers at 24 HAT*	*Total females at 24 HAT*
Egg treatment[a]	*Dead 96 HAT (%) (±SE)*[c,d]	*Dead 96 HAT (%) (±SE)*[c]	*Dead 96 HAT (%) (±SE)*[c]
Treated[b] eggs; treated filter paper	70.4 (±12.4) a,1	19.1 (±4.0) a,2	41.7 (±3.2) a
Untreated eggs; treated filter paper	14.8 (±5.6) b,1	7.1 (±2.5) b,1	14.3 (±2.5) b
Untreated control	0 c,1	0 c,1	0 c

[a] $n = 4$.
[b] Treated with indoxacarb at 0.01 kg AI ha^{-1}.
[c] Means within a column followed by the same lower case letter are not significantly different ($P > 0.05$; LSD) between treatments.
[d] Means within a row followed by the same number are not significantly different ($P > 0.05$; *t*-test) between females that did and did not eat eggs at 24 HAT, but were dead at 96 HAT.

4 CONCLUSIONS

These studies demonstrate that *L lineolaris* and *G punctipes* respond very similarly to the indoxacarb SC formulation after normal toxicological routes of administration, including topical treatment, prolonged tarsal contact to treated plants, or by feeding on treated plants. Clearly both species are most sensitive to orally administered indoxacarb via feeding on treated cotton plants, and this is probably the major route of intoxication of *L lineolaris* in the field. We also found that indoxacarb residues appeared to be more insecticidally active when the treated cotton plants were held at a higher relative humidity; this may be due to an effect on the plant cuticle which makes the compound more orally bioavailable to the *L lineolaris*. This insect, an increasingly important pest in US cotton, is well controlled in the field by indoxacarb at labelled use rates, and it is important to be able to understand the major mechanism of intoxication.

When poisoned insects were analyzed by HPLC–mass spectrometry, we observed substantial concentrations of both parent indoxacarb and the decarbomethoxylated metabolite DCMP, though the conversion was much slower than had been observed in Lepidoptera. Since it has previously been shown that DCMP is active in blocking lepidopteran sodium channels, whereas indoxacarb is not,[2,3] it is likely that bioactivation to this metabolite is the toxic mechanism of action in *L lineolaris* and in *G punctipes* as well. After topical application *L lineolaris* and *G punctipes* show very similar dose-mortality responses; however, at the same topical dose *G punctipes* accumulated significantly larger amounts of both DCMP and indoxacarb, especially the latter. Apparently *G punctipes* is more tolerant to these compounds and may perhaps sequester the compound away from nervous tissue. The level of toxin uptake that occurred in these tests is probably much larger than the level of toxin than would occur via natural uptake of field-applied indoxacarb SC.

When dead *L lineolaris* and *G punctipes* were analyzed by HPLC–MS after feeding on sprayed cotton plants, both species showed strikingly similar levels of DCMP accumulated in their bodies. Thus it appears that lethality is likely to ensue when the animal is able to bioactivate indoxacarb to give *c* 0.15 ng DCMP mg^{-1} body weight, no matter what the initial ingested dose of indoxacarb had been. When this level of compound is compared with the topical toxicity to both species (LD_{50} 35 ng per insect), it is clear that dermal penetration into both insects is relatively inefficient as a mode of intoxication. When these data are compared with previous studies, it is clear that these hemipterans bioactivate indoxacarb less efficiently and are less sensitive on a per weight basis than many Lepidoptera.[2,3]

No differential susceptibility occurred between topical, tarsal contact and foliar feeding tests for *L lineolaris* and *G punctipes*. Both the pest and predator probably would be intoxicated after feeding on plant residues of indoxacarb in fields with high humidity. However, earlier field studies with indoxacarb under humid conditions have demonstrated that this insecticide did not adversely affect populations of *G punctipes*.[11] We have found that when female *G punctipes* ate indoxacarb-treated *H zea* eggs, there was significant toxicity. However, only *c* 15% of the females consumed the treated eggs, and a significant diminution of feeding was observed in response to the insecticide for the rest of the females. Even those females that appeared to suffer this feeding inhibition after exposure to indoxacarb-treated eggs for 24 h recovered their appetite for untreated eggs by 96 h after the treated eggs were withdrawn. In addition, a preliminary laboratory study on feeding behavior of *G punctipes* females has shown that the females spend only *c* 20% of their total feeding time feeding on plant tissue, and 80% of their feeding time on insect eggs, when these latter are readily available (Tillman, PG, unpublished). Thus, their feeding behavior, in addition to the fact that very few females consume indoxacarb-treated eggs, may explain the survival of *G punctipes* in indoxacarb-treated fields. Moreover, *G punctipes* may even avoid contact with indoxacarb in the field by consuming any of many different prey species that may be indoxacarb-free. In addition, eggs of this predator on the underside of cotton leaves could be protected from the insecticide. Further laboratory and field studies are necessary to understand the observed survival of *G punctipes* in indoxacarb-treated fields.

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