TOXICITY OF INDOXACARB™ TO THE TARNISHED PLANT BUG, *LYGUS LINEOLARIS* (HEMIPTERA: MIRIDAE), AND THE BIG-EYED BUG, *GEOCORIS PUNCTIPES* (HEMIPTERA: LYGAEIDAE)

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INTRODUCTION

Indoxacarb (Steward™ is a new oxadiazine insecticide discovered by the E. I. DuPont Co that has shown outstanding field insecticidal activity, environmental compatibility, and safety to nontarget organisms (Wing *et al*., 2000). Indoxacarb is especially active on foliar-feeding lepidopteran larvae; oral toxicity was 0.01 and 0.03 ng/mg body weight for *Heliothis virescens* (F.) and *Spodoptera frugiperda* (Smith), respectively (Wing *et al*., 2000). 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 (Wing *et al*., 1998). One of the formulations of indoxacarb is a 14.5% suspensible concentrate with the trade name Steward™in the United States.

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is a pest in cotton in the southeastern United States (Hanny *et al*., 1977). *Lygus lineolaris* may become a critical pest in this region with the widespread planting of *Bt* cotton and the successful eradication of the boll weevil (*Anthonomus grandis grandis* Boheman). Potential development of insecticide-resistant populations of tarnished plant bug is of major concern because of the growing focus on this pest as a target for insecticide treatment and the paucity of alternative insecticides (Snodgrass, 1996). The big-eyed bug, *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae), is a predator of many pest species, including eggs and small larvae of *H. virescens* and *Helicoverpa zea* (Boddie) (Lingren *et al*., 1968). *Geocoris punctipes* also feeds on plants, increasing the likelihood of its survival during the absence of invertebrate hosts (Eubanks and Denno, 1999). Indoxacarb is highly efficacious against *L. lineolaris* and may be a new tool in an integrated pest management program for this pest in cotton (Teague *et al*., 2000). 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 (Teague *et al*., 2000). However, *G. punctipes* were not affected adversely by applications of indoxacarb in small field plot tests (Ruberson and Tillman, 2000).

The specific objectives of our study were to determine critical mechanisms of intoxication of *L. lineolaris* after indoxacarb treatment and 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 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.

MATERIALS AND METHODS

Insects

Lygus lineolaris and *G. punctipes* adult females were collected using a sweep net from flowering wild mustard in Tifton, Georgia. Eggs of *H. zea* were obtained from a colony reared in the USDA, ARS rearing laboratory at Tifton, Georgia, on an agar soybean flour-wheat germ diet at 26 °C, 50 ± 5% RH, and a photoperiod of 15:9 L:D h (Perkins *et al*., 1973).

Topical Bioassays

Steward™was provided by DuPont Agricultural Products, Wilmington, Delaware. Indoxacarb application solutions were prepared using water as the diluent. The concentrations in µg per ml are corrected for percent active ingredient. A group of five adult insects in Petri dishes were anesthetized with CO_2 for ca 45 s. Next, a 0.2 µl droplet of insecticide or water control was placed on the dorsal thorax of each anesthetized insect using a microapplicator. Each treated insect was fed and held in a Petri dish 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 14 times. Topical toxicity data were analyzed by the SAS PROBIT procedure (SAS Institute, 2000). LD_{50}s were considered significantly different if the 95% confidence limits did not overlap.

Tarsal Contact Bioassays

Serial dilutions of indoxacarb 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. A Potter spray tower with air pressure at 1.47 x 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, then were removed to clean Petri dishes without cotton leaves. All treated insects were then fed 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, 2000). LC_{50}s were considered significantly different if the 95% confidence limits did not overlap.

Feeding Bioassays

General procedures. Greenhouse-grown cotton plants were 7-9 weeks old when used in experiments. Plants, except for controls, were sprayed with indoxacarb at a rate of 0.1 kg ai ha^{-1} using a two-row spray boom calibrated to deliver 112.25 liter per ha using a TX-8 nozzle at an air pressure of 2.75 x 10^5 Pa and a speed of 1.86 km per h. The final concentration was thus 890 µg indoxacarb active ingredient per ml spray suspension. The height of the nozzle above the plant was ca 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 d, and 5 d 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 (twice in 20 ml fresh water each time) for 30 s using a 50-ml disposable polypropylene centrifuge tube. For the detergent wash, the clipped terminal was washed in 10% liquid detergent (Dawn®) for one minute. 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 cups were placed individually in a cardboard carton or plastic cylinder. The carton was a 3.8 liter 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. Once cages with plants were prepared, 20 insects of either species were placed in each cage. Mortality for each species was recorded 24, 48, and 72 h after treatment.

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. The plant tissue was washed by swirling in water (twice in 10 ml fresh water each time) for 15 s in a 50 ml centrifuge tube. 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, 2000), followed by least significant difference (LSD) separation of means.

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 (ca 52% RH) and plastic cylinder (ca 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 thermohygrometer. Relative humidity and percentage mortality data were compared between treatments by the SAS ANOVA procedure, followed by LSD separation of means (SAS Institute, 2000). Percentage mortality data were compared between species using *t*-tests.

Test 3. Effect of Detergent Washes on Toxicity of Sprayed Cotton Plants. The three treatments included unwashed, detergent-washed, and control terminals in plastic cylinder cages; only *L. lineolaris* was tested. Percentage mortality data were analyzed by the SAS ANOVA procedure followed by LSD separation of means (SAS Institute, 2000).

Geocoris punctipes Treated-Egg Bioassay

After eating at least five *H. zea* eggs, *G. punctipes* females were starved overnight and then aspirated singly into experimental Petri dishes. The three experimental treatments were (1) 20 *H. zea* eggs (treated) on filter paper treated with indoxacarb at 0.1 kg a.i. per ha (0.09 lbs a.i. per acre), (2) 20 untreated *H. zea* eggs on filter paper treated with indoxacarb 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 at the rate of 23 Gy per min (± 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 eggs. So eggs were placed on very small (2.3 cm in diameter) filter paper 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 at an air pressure of 20 psi. After spraying, treated filter paper with or without eggs was placed in a clean Petri dish free of residues of indoxacarb. Filter paper and eggs were allowed to dry for 1 h before aspirating females individually into Petri dishes. 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 ca 27 °C and 50-60% RH. Data on percentage of females that consumed eggs were determined 24, 48, 72, and 96 "hours after treatment"(HAT) and compared by the ANOVA procedure followed by LSD separation of means (SAS Institute, 2000). New unsprayed eggs (20 *H. zea*/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 (SAS Institute, 2000).

RESULTS AND DISCUSSION

Topical Bioassays

The topical susceptibility of *L. lineolaris* and *G. punctipes* to indoxacarb was very similar (Table 1). The LD_{50} for both insects was approximately 35 ng indoxacarb a.i. per insect.

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

Hours after treatment	Species	n	Slope (SE)	LD50 (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] Lethal doses of insecticide in ng per insect at the LD50 level of probit mortality (with 95% confidence interval). Range of percentage control mortality was 0-5.0% and 1.7-6.7% for G. *punctipes* and *L. lineolaris*, respectively. Average body weight is 6.5 mg for *L. lineolaris* and 4.0 mg for *G. punctipes.*

Tarsal Contact Bioassays

Neither *L. lineolaris* nor *G. punctipes* were affected by walking on dried residues of indoxacarb on cotton plants, with LC_{50}s of > 3,000 µg indoxacarb a.i. per ml (Table 2). Apparently tarsal contact is an inefficient mode of uptake.

Table 2. Toxicity of indoxacarb-treated cotton leaves to adult *Lygus lineolaris* and *Geocoris punctipes* by tarsal contact 48 h after treatment.

Species	n	Slope (SE)	LC50 (95% CI)[a]
G. punctipes	200	1.86 (0.36)	3782 (2813->4800)
L. lineolaris	200	2.94 (0.52)	3999 (3250->4800)

[a] Lethal doses of indoxacarb (µg a.i. per ml) at the LC50 level of probit mortality (with 95% confidence interval). Percentage control mortality was 10.0% and 0% for G. *punctipes* and *L. lineolaris*, respectively.

Feeding Bioassays

Test 1. Effect of water washes on toxicity of sprayed cotton plants. The mortality observed for both species after feeding through dried indoxacarb residues on cotton terminals was similar for all treatments (Table 3). Unwashed terminals led to approximately 90% mortality at 0 d, and 50% mortality at 5 d, for both species. The insecticide rates used in this test compare well with field-use rates of indoxacarb; the sprayed rate of 0.1 kg a.i. per ha at 112.25 litre per ha is approximately 890 µg a.i. per ml. Previous laboratory bioassays have shown that indoxacarb was active against *L. lineolaris* that fed on this insecticide on an inert matrix (Teague *et al*., 2000). The water-washed cotton terminals still retained insecticidal activity, though significantly less than that of unwashed plants. Interestingly, the insecticidal activity difference between unwashed and water-washed plants became more pronounced at the later days after application. The activity in washed plants is probably due to indoxacarb that has penetrated the cuticle and is refractory to water washout. Very few insects were dead in the wash treatment for 5 d after treatment; however, unwashed plants still showed excellent insecticidal activity, which is an indication of the longevity of indoxacarb in cotton fields.

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

		Mortality (%)(Mean ± SE)[b]	
Days after treatment	Wash treatment[a]	*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 72 h 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.

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 compared with the low humidity environment (Table 4). There was no statistical difference ($F = 0.28$; df = 44; $P = 0.7594$) in temperature between the plant cages: mean (SE) cylinder temperature was 22.43 °C (0.19), mean (SE) carton temperature was 22.34 °C (0.19), and mean (SE) control temperature was 22.26 °C (0.13). These sucking insects are thus capable of absorbing and bioactivating indoxacarb after oral administration, but they do so more slowly than the Lepidoptera (Wing *et al*., 2000).

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

Days after treatment	Treatment[a]	Mortality (%) (Mean ± SE)[c]		
		% RH[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 72 h 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.

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 5). 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 cells in a form that is 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.

Geocoris punctipes Treated-Egg Bioassay

Indoxacarb significantly reduced the percentage of *G. punctipes* females that consumed eggs 24 h after treatment (HAT) (Table 6), indicating that either *G. punctipes* females were repelled by indoxacarb, or this insecticide worked 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 some 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 96 h.

Geocoris punctipes feeds by inserting the proboscis into an egg and sucking the inside of the egg into the digestive tract. The observed reduction in feeding could be attributed to the toxicity of the N-decarbomethoxyllated metabolite (DCMP) of indoxacarb in *H. zea* eggs. Treated eggs of *H. zea*

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

Days after treatment	Wash treatment[a]	Mortality (%) *L. lineolaris* (Mean ± 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 72 h after treatment. Means followed by the same letter are not significantly different (P > 0.05; LSD) between wash treatments for a single day.

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 probably was 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 of indoxacarb in the filter paper treatment was 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 better understand 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 (nonconsumers) was examined 96 h after treatment (HAT) (Table 7). For consumers and nonconsumers, mortality was highest for treated eggs, lower for untreated eggs on treated filter, and nonexistent for the untreated control. Even though the nonconsumers 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 indoxacarb. Mortality was similar for consumers and nonconsumers for the filter paper treatment, so mortality was due only to probing treated filter paper. Mortality was much higher for consumers than nonconsumers for the treated eggs, suggesting that females that ate eggs ingested more indoxacarb than nonconsumers. 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

Table 6. Percentage of *G. punctipes* females consuming eggs 24, 48, 72, and 96 h after treatment (HAT) for three egg treatments.

Hours after treatment	Egg treatment[a]	Females consuming eggs (%) (Mean ± SE)[c]
24	Treated[b] 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] n = 4.
[b] Treated with indoxacarb at 0.01 kg ai ha-1.
[c] Means within a column followed by the same lowercase letter are not significantly different (P > 0.05; LSD) among treatments for a single HAT category.

attributed to probing treated filter, and thus mortality due only to consuming treated eggs probably was less than could be assessed or ca 20%. Even so, the mortality of *G. punctipes* females was moderately low when these females were given eggs treated with indoxacarb.

SUMMARY

These studies demonstrate that *L. lineolaris* and *G. punctipes* respond very similarly to indoxacarb 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 feeding on indoxacarb-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's cuticle that makes the indoxacarb more orally bioavailable to the *L. lineolaris*. This insect, an increasingly important pest in U.S. 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.

No differential susceptibility occurred in 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* (Ruberson and Tillman, 2000). We have found that when female

Table 7. Mortality 96 h after treatment (HAT) for *G. punctipes* females that consumed (consumers) and did not consume eggs (nonconsumers) 24 h after being exposed to three egg treatments.

Egg treatment[a]	Consumers at 24 HAT	Non-consumers at 24 HAT	Total females at 24 HAT
	% dead 96 HAT(Mean ± SE)[c,d]	% dead 96 HAT(Mean ± SE)[c]	% dead 96 HAT(Mean ± 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 lowercase 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.

G. punctipes ate indoxacarb-treated *H. zea* eggs, there was significant toxicity. However, only ca 15% of the females consumed the indoxacarb-treated eggs, and a significant reduction 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 indoxacarb-treated eggs were withdrawn. Also, a preliminary laboratory study on feeding behavior of *G. punctipes* females has shown that the females spend only ca 20% of their total feeding time feeding on plant tissue, and 80% of their feeding time on insect eggs, when these are readily available (P. G. Tillman, unpub.). 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. Also, *G. punctipes* may even avoid contact with indoxacarb in the field by consuming any of many different prey species that may be indoxacarb-free. Also, eggs of this predator on the underside of cotton leaves could be protected from contact with indoxacarb. Further laboratory and field studies are necessary to understand the observed survival of *G. punctipes* in indoxacarb-treated fields.

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