Contact Toxicity of Selective Insecticides for Non-Target Predaceous Hemipterans in Soybeans¹

M. E. Baur,² J. Ellis,^{3,4} K. Hutchinson³ and D. J. Boethel

Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803 USA

Abstract New insecticides with selectivity for lepidopterous pests are currently being registered for use in soybeans throughout the mid-south. The effect of these products on important hemipteran beneficials found in Louisiana soybean agroecosystems was tested in the laboratory and the field. In the laboratory, adult and nymphal hemipteran predators were exposed to foliage treated with selective insecticides (methoxyfenozide and indoxacarb) or broad-spectrum pyrethroid and organophosphate insecticides, and survival between these two groups was compared using contingency table analysis. Survival of Geocoris punctipes (Say), Tropiconabis capsiformis (Germar), or Podisus maculiventris (Say) adults and nymphs exposed to a rate of 0.12 kg Al/ha of indoxacarb or a rate of 0.22 kg Al/ha of methoxyfenozide was consistently higher than their survival when exposed to 0.03 kg Al/ha of lambda-cyhalothrin or 0.84 kg Al/ha of acephate. At the rates tested, indoxacarb reduced the survival of P. maculiventris adults and nymphs more than methoxyfenozide. In the field, indoxacarb at 0.05 and 0.07 kg Al/ha significantly reduced G. punctipes populations at 2 and 7 days after treatment compared to the untreated check. Methomyl or thiodicarb, both at 0.5 kg Al/ha, also significantly reduced populations of T. capsiformis and Nabis rosiepennis (Reuter) 2 days after treatment. None of the other treatments tested in the field (methoxyfenozide, spinosad, and permethrin) significantly reduced predator populations. The results from this study indicate that both methoxyfenozide and indoxacarb will affect hemipteran predator populations less than pyrethroid, organophosphorus, or carbamate insecticides. Of the two, however, indoxacarb may affect predators more than methoxyfenozide.

Key Words Geocoris punctipes, Tropiconabis capsiformis, Podisus maculiventris, indoxacarb, methoxyfenozide, Geocoridae, Pentatomidae, Nabidae

An arsenal of new insecticides is becoming available to the soybean integrated pest management practitioner. Many of these newer products are selectively toxic to target pests. Applications of these selective insecticides against lepidopteran pests should have minimal impact on predaceous and parasitic arthropods (Boyd and Boethel 1998a, b, Kunkel et al. 2001, Elzen 2001, Bajwa and Aliniazee 2001). The insect growth regulators (e.g., diflubenzuron) have no effect on spiders (Bajwa and Aliniazee 2001) or encrytid parasitoids (Willrich and Boethel 2001), but diflubenzuron was toxic to *Podisus maculiventris* (Say) when imbibed (De Clercq et al. 1995). Insecticides based on *Bacillus thuringiensis* Berliner have no activity on predaceous species

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²Address requests to M. Baur (mbaur@agctr.lsu.edu).

³Department of Agronomy.

⁴Current address: Bayer Crop Science, 206 Kennedy Flat Rd, Leland, MS 38746.

(Boyd and Boethel 1998a,b). Spinosad and emamectin benzoate affect predator survival, but to a lesser extent than pyrethroids or carbamates (Boyd and Boethel 1998a, b, Elzen 2001). Thirty-seven percent of *Orius insidiosus* (Say) survived exposure to indoxacarb in the field, and almost 82% survived exposure to spinosad (Studebaker and Kring 1999). In Texas alfalfa, *Medicago sativa* L., spinosad reduced nabid populations significantly, but *Geocoris* spp. populations were unaffected (Muegge and Friesen 2000). Although the newer products as a whole are less disruptive toward natural enemy populations, some may have a negative effect. Because hemipteran predators are among the most important natural enemies in the soybean agroecosystem (Turnipseed and Kogan 1983), the present study examined the effect of two selective insecticides being considered for registration in soybeans on the predominant hemipteran predators in the soybean agroecosystem in Louisiana.

Populations of predators could be affected in several ways by selective insecticides (Croft and Brown 1975). Sublethal effects such as reduced longevity, fecundity, or feeding have been demonstrated with several generalist predator species (Mohaghegh et al. 2000, Elzen 2001). Fipronil treatment of Heliothis zea (Boddie) eggs reduced O. insidiosus feeding by 50% and fecundity by more than 95% (Elzen 2001). Similarly, fipronil and imidacloprid treatment of H. zea eggs reduced G. punctipes feeding by about 50% (Elzen 2001). When P. maculiventris imbibe teflubenzuron, the duration of the last two nymphal stages increased by 5% primarily because the last (fifth) stadium was lengthened by 10% (Mohaghegh et al. 2000). In addition, P. maculiventris egg hatch was reduced by 17% when females imbibed teflubenzuron (Mohaghegh et al. 2000). Predators may emigrate from treated fields because of lower prey density, or predator density could decline because the lower prey density causes a subsequent increase in intraguild predation (Rosenheim et al. 1995, Ruberson et al. 1998). Several routes of entry into the predator hemocoel could also cause direct toxicity. Predators could feed on intoxicated prey (Boyd and Boethel 1998b), or be exposed to either insecticide residues on prey items (Elzen 2001) or to residues on foliar surfaces. The present study focused on acute toxicity to adults and nymphs caused by direct contact with insecticides on foliar surfaces and the possible disruption of the molting process of nymphs caused by these products.

Materials and Methods

Laboratory bioassays. The following formulated insecticides were used for the laboratory bioassays at the following rates: lambda-cyhalothrin (Karate Z® 2.09 SC, 0.028 kg Al/ha, Syngenta Corp., Wilmington, DE); methoxyfenozide (Intrepid® 80 WP, 0.224 kg Al/ha, Dow Agrosciences, Indianapolis, IN); acephate (Orthene® 90 S, 0.84 kg Al/ha, Valent USA Corp., Walnut Creek, CA); and indoxacarb (Steward® 1.25 SC, 0.12 kg Al/ha, DuPont, Wilmington, DE). In addition to indoxacarb and methoxyfenozide, the following insecticides were used in the field trials at the rates listed in Table 2: thiodicarb (Larvin® 3.2 F, Aventis, Research Triangle Park, NC), permethrin (Ambush® 2 EC; Syngenta Corp., Wilmington, DE), methomyl (Lannate® 2.4 EC, DuPont, E.I. Nemours & Inc., Wilmington, DE), and spinosad (Tracer® 4 F, Dow Agrosciences, Indianapolis, IN).

Adults and last-instar nymphs of *G. punctipes* (Geocoridae), *P. maculiventris* (Pentatomidae), and *Tropiconabis capsiformis* (Germar) (Nabidae) were collected from soybean fields in Bossier, Iberville, and East Baton Rouge parishes in Louisiana. These were brought to the laboratory and held for use in bioassays. Soybean fields in the soybean-cotton-corn agroecosystem in Bossier Parish are more intensively managed with insecticides, and the soybean fields in the soybean-sugarcane agroecosystem in the Iberville and East Baton Rouge parishes are less intensively managed with insecticides.

Insecticide treatments were applied on the mornings of 4 and 11, Aug 1999 and 11, 16, and 25 July 2000 at the St. Gabriel Research Station (Iberville Parish) to the soybean cultivar Pioneer 97B61 using a CO_2 pressurized backpack sprayer calibrated to deliver 140 Ls/ha. Plots were 6 rows by 15.2 m and soybean rows were 0.9 m apart. Foliage was collected 12 h, and 1, 2, and 5 d after treatment, and only moderately sized leaves from the top of the plant canopy were picked for use in bioassays. Trifoliates were returned to the laboratory, and leaflets were separated and placed in 9-cm Petri dishes lined with moistened filter paper.

Geocoris punctipes and T. capsiformis adults and nymphs were placed in the Petri dishes individually with a treated leaflet and 15 frozen Pseudoplusia includens (Walker) eggs. The presence of prey eggs substantially increased the survival of T. capsiformis in the control treatments over the 72 h period and also ensured that nymphs of both species would undergo a molt during the bioassay. By including a nymphal molt, we hoped to assess any effect methoxyfenozide might have on molting predaceous hemipteran nymphs. Methoxyfenozide can disrupt the molting process in holometabolous insect larvae. Pseudoplusia includens eggs were provided to G. *punctipes* to ensure that assay conditions were consistent across species; the presence of food did not significantly increase G. punctipes survival in the control treatments (data not shown). Podisus maculiventris adults and nymphs were supplied with a third-instar (20 to 30 mg weight) P. includens larva. Before placement in Petri dishes, *P. includens* larvae were stunned by pinching the heads between the forefinger and thumb using moderate pressure, and being careful not to rupture the head capsule. Larvae treated in this manner were still attractive as prey for the P. maculiventris adults and nymphs but would not feed on the treated foliage during the bioassay period. This treatment again ensured that the P. maculiventris nymphs would undergo a molt during the bioassay period. Each experiment consisted of five replicates (Petri dishes) for each insect stage (adult and nymph) by treatment combination. Petri dishes were held at ambient laboratory conditions ($23 \pm 2^{\circ}$ C, and 50%) RH).

Mortality was checked at 24, 48 and 72 h after placement of the predators in the Petri dishes. Predators were scored as dead if they did not respond to gentle prodding using a probe. Few insects (<1%) were ataxic and were observed staggering around the Petri dish by the end of the bioassay; these were scored as living. Although the assays were checked 24, 48 and 72 h after predator placement in the Petri dishes, only the data from the 72 h evaluation are presented because nymphs had completed a molt within this time, and control mortality remained less than 10%.

The association between predator survival and insecticide treatment was analyzed by three (one table for each predator species) 5×2 contingency tables where columns represented the number of live and dead predators and rows represented the five insecticides. Data were summed over the four samples taken from treated plots in the field (12 h, and 1, 2 and 5 d after treatment). In a second set of contingency tables, we examined if the effect of the insecticides on predator survival diminished with increasing exposure of insecticides to field conditions (from 12 h to 5 d). The procedure PROC FREQ in the Statistical Analysis Software (SAS Institute 1994) was used to calculate the continuity-adjusted chi-square for $s \times r$ tables. The continuityadjusted chi-square was used because of the low number of deaths in three (check, indoxacarb, and methoxyfenozide) of the five treatments, and therefore the low cell counts in the "number dead" column. When cell counts increase, the continuity-adjusted chi-square behaves like the Pearson chi-square (SAS Institute 1994).

Field trials. Two, parallel small plot trials were conducted in 1999 at the Red River Research Station, Bossier Parish. Treatments were applied to Asgrow 6101 soybeans in the R5 growth stage (Fehr et al. 1971) with a high-clearance CO_2 sprayer calibrated to deliver 93.5 L/ha at 4.2 Kg/cm² through TeeJet TXVS-6 hollow cone nozzles (2/row). Plots were 15.2 m by 4 rows (0.99 m centers) and arranged in randomized complete block design with 4 replications. One 25-sweep sample per plot was taken with a standard 0.38-m diam sweep net at 2 and 7 d after treatment. Data were analyzed by ANOVA and means separated using Tukey's HSD (Snedecor and Cochran 1980). Insecticides and rates used are listed in Table 2.

Results and Discussion

Laboratory results. Survival of predators exposed to indoxacarb and methoxyfenozide was significantly higher than for those exposed to two broad-spectrum insecticides currently recommended for use in soybeans (pyrethroids and organophosphates) (Table 1). Both lambda-cyhalothrin and the acephate are labeled for use against lepidopteran pests, but these products are primarily recommended for control of coleopteran defoliators and phytophogous heteropteran pests in Louisiana soybeans (Baldwin et al. 2001). Therefore, these products were chosen as positive controls and were expected to be detrimental to the hemipteran predators. Lambdacyhalothrin was more toxic to *T. capsiformis* than acephate. The opposite was true for *G. punctipes* and *P. maculiventris;* acephate reduced survival more than lambdacyhalothrin. Acephate is more effective than lambda-cyhalothrin for control of herbivorous pentatomids in the genus *Euschistus* (Fitzpatrick et al. 2001a,d); whereas, both products are equally effective against the more susceptible pentatomid, *Nezara viridula* (L.) (Fitzpatrick et al. 2001a,d).

Methoxyfenozide can act as an ecdysone agonist that disrupts the molting process in holometabolous insect larvae (Dhadialla et al. 1998, Oberlander et al. 1998), but it did not affect the molting process in the hemimetabolous hemipteran predators studied here. Seventy-eight percent of the *T. capsiformis* nymphs survived exposure to methoxyfenozide compared to 84% of the adults, and the same trend was observed for indoxacarb. Methoxyfenozide was no more toxic to *P. maculiventris* nymphs than adults, and more *G. punctipes* nymphs survived exposure than adults.

Tropiconabis capsiformis adults and nymphs were the most susceptible to insecticide exposure of the three predators studied (Table 1). Both indoxacarb and methoxyfenozide lowered adult and nymph *T. capsiformis* survival significantly compared to the check treatment.

Survival of the pentatomid predator, *P. maculiventris,* was lowest of the three hemipteran predators when exposed to indoxacarb (Table 1). In the popular literature (Delta Farm Press), it has been suggested that indoxacarb may suppress populations of phytophagous pentatomids (stink bugs) and mirids (plant bugs) (Hollis 2001). The data presented here appear to indicate that indoxacarb may affect the predaceous pentatomids to a greater extent than the predaceous geocorids and nabids. However, in the laboratory bioassays we used a rate of indoxacarb that is higher than the rates currently under investigation for control of *P. includens* (0.0616-0.1232 kg Al/ha)

							Overall
Predator	Insecticide	Ν	12h	1d	2d	5d	survival
G. punctipes	methoxyfenozide	25	24 (96)	25 (100)	20 (80)	20 (80)	89
Adults	indoxacarb	25	24 (96)	23 (92)	23 (92)	19 (76)	89
	L-cyhalothrin	25	20 (80)	15 (60)	17 (68)	17 (68)	69
	acephate	25	13 (52)	17 (68)	16 (64)	14 (56)	60
	Check	25	25 (100)	25 (100)	23 (92)	24 (96)	97
Chi-square	(4 df)		30**	23**	9 NS	12*	57**
G. punctipes	methoxyfenozide	25	20 (80)	25 (100)	23 (92)	23 (92)	91
Nymphs	indoxacarb	25	25 (100)	25 (100)	23 (92)	25 (100)	98
	L-cyhalothrin	25	23 (92)	20 (80)	21 (84)	21 (84)	85
	acephate	25	5 (20)	9 (36)	15 (60)	17 (67)	45
	Check	25	25 (100)	24 (96)	25 (100)	25 (100)	99
Chi-square	(4 df)		46**	11*	8 NS	12*	65**
T. capsiformis	methoxyfenozide	20	17 (85)	18 (90)	14 (70)	18 (90)	67 (84)
Adults	indoxacarb	20	15 (75)	16 (80)	18 (90)	19 (95)	68 (85)
	L-cyhalothrin	20	4 (20)	4 (20)	5 (25)	11 (55)	24 (30)
	acephate	20	8 (40)	9 (45)	10 (50)	15 (75)	42 (52)
	Check	20	20 (100)	19 (95)	18 (90)	19 (95)	76 (95)
Chi-square	(4 df)		26**	37**	27**	15**	98**
T. capsiformis	methoxyfenozide	20	10 (50)	16 (80)	18 (90)	18 (90)	62 (78)
Nymphs	indoxacarb	20	20 (60)	15 (75)	17 (85)	14 (70)	58 (73)
	L-cyhalothrin	20	0 (0)	2 (10)	9 (45)	13 (65)	24 (30)
	acephate	20	9 (45)	8 (40)	12 (60)	14 (70)	43 (54)
	Check	20	20 (100)	18 (90)	20 (100)	19 (95)	77 (95)
Chi-square	(4 df)		40**	38*	21**	8 NS	89**
P. maculiventris	methoxyfenozide	20	16 (80)	20 (100)	17 (85)	20 (100)	73 (91)
Adults	indoxacarb	20	12 (60)	11 (55)	15 (75)	20 (100)	58 (73)
	L-cyhalothrin	20	16 (80)	16 (80)	16 (80)	13 (65)	61 (76)
	acephate	20	9 (45)	7 (35)	6 (30)	11 (55)	33 (41)
	Check	20	20 (100)	20 (100)	20 (100)	20 (100)	80 (100)
Chi-square	(4 df)		18**	30**	32**	29*	89**
P. maculiventris	methoxyfenozide	20	16 (80)	17 (85)	20 (100)	20 (100)	73 (91)
Nymphs	indoxacarb	20	11 (55)	12 (60)	20 (100)	20 (100)	63 (79)
	L-cyhalothrin	20	8 (40)	15 (75)	20 (100)	20 (100)	63 (79)
	acephate	20	3 (15)	4 (20)	9 (45)	16 (80)	32 (40)
	Check	20	20 (100)	20 (100)	20 (100)	20 (100)	80 (100)
Chi-square	(4 df)		36**	34**	16**	49**	97**

Table 1. Survival (and percentage survival) of predators (either adults or last instar nymphs) after 72 h of exposure to insecticide-treated soybean foliage taken from the field 12h, 1d, 2d, or 5d after treatment. Chisquare values and associated probabilities (with 4 df) are also shown

Significant chi-square values are highlighted in boldface, and levels of significance indicated by asterisks; NS indicates P > 0.05, *indicates P < 0.05, and **indicates P < 0.01. N indicates number of insects tested.

(Fitzpatrick et al. 2001c,e) or Anticarsia gemmatalis Hübner (0.028 kg Al/ha) (Fitzpatrick et al., 2001b) in Louisiana soybeans. Lower rates of indoxacarb should have less impact on predaceous pentatomids.

Field results. In the field trials (combined), there were significant reductions in predator populations in plots treated with indoxacarb, thiodicarb, and methomyl com-

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tenozide),	Steward (Indox	acarb)]*				
		Rate**	Nabids		Geocoris	punctipes
Insecticide		Kg Al/ha	2 DAT	7 DAT	2 DAT	7 DAT
			Trial I			
Indoxacarb	1.25 SC	0.050	1.8 ± 0.5 ab***	4.8 ± 1.8	$5.3 \pm 1.0 \text{b}$	$5.8 \pm 0.9 c$
Indoxacarb	1.25 SC	0.073	3.3 ± 0.9 ab	3.5 ± 1.4	$5.3 \pm 1.3 \mathrm{b}$	6.8 ± 1.0 bc
Thiodicarb	3.2 F	0.504	1.8 ± 0.5 ab	2.5 ± 0.8	$7.0 \pm 1.6 b$	10.8 ± 1.5 a
Methomyl	2.4 EC	0.504	0.0 ± 0.0 b	1.8 ± 0.8	8.5 ± 1.6 ab	7.5 ± 1.5 bc
Spinosad	4 F	0.056	2.5 ± 0.9 ab	2.0 ± 0.4	7.8 ± 1.9 ab	7.8 ± 1.1 abc
UTC	1		5.0 ± 0.3 a	6.5 ± 3.0	14.3 ± 2.7 a	9.5 ± 0.8 ab
			Trial II			
Methoxyfenozide	80 WP	0.140	1.0 ± 0.7 a	3.8 ± 0.2	6.8 ± 1.3	8.8 ± 0.9
Methoxyfenozide	80 WP	0.070	0.8 ± 0.2 a	2.8 ± 0.5	5.8 ± 1.2	9.3 ± 3.0
Indoxacarb	1.25 SC	0.056	3.3 ± 2.0 ab	6.0 ± 1.2	3.3 ± 1.2	7.0 ± 1.6
Thiodicarb	3.2 F	0.504	0.0 ± 0.0 b	0.8 ± 0.2	12.3 ± 3.3	10.0 ± 1.8
Permethrin	2.0 EC	0.112	1.3 ± 0.7 a	4.0 ± 0.9	3.0 ± 0.6	7.8 ± 2.6
Spinosad	4 F	0.099	4.0±2.1 a	2.8 ± 0.5	4.5 ± 1.3	9.0 ± 1.7
UTC			2.4 ± 0.9 a	2.0 ± 0.9	6.5 ± 1.2	15.0 ± 3.4

* Counts were taken with standard 0.38 m diameter sweep nets and 25 sweeps per sample.

** Units are kg active ingredient per hectare.

*** Different letters following values within a column indicate significant differences.

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pared to the untreated check (UTC). (Table 2). In plots treated with thiodicarb or methomyl, nabid populations were significantly reduced 2 days after treatment (2 DAT) (F = 4.7 and 6.6; df = 3, 21 and 30; P < 0.02). Geocoris punctipes populations were significantly reduced 2 (2 DAT) and 7 days after treatment (7 DAT) in indoxacarb treated plots in the first trial (F = 4.0 and 4.6; df = 3, 21; P < 0.02). No statistically significant reductions in predator populations were observed in plots treated spinosad, methoxyfenozide, or permethrin compared to the untreated check. These results indicate that indoxacarb may reduce predator populations to a greater extent than methoxyfenozide and to a similar or lesser extent than thiodicarb and methomyl.

Indoxacarb is a oxadiazine that acts as a stomach poison (Wing et al. 1998) and was toxic to some of the predaceous hemipterans studied here. Indoxacarb has also been shown to reduce *O. insidiosus* populations in the field (Studebaker and Kring 1999). Methoxyfenozide blocks potassium channels (Salgado 1992), but it did not appear to be particularly toxic to nymphs or adults of any of the hemipteran predators studied here. Our data, combined with data published by others, demonstrate that the newer selective insecticides, developed as part of the integrated management strategy, have less acute toxicity than the broad-spectrum insecticides of the past (Hamilton and Lashomb 1997, Boyd and Boethel 1998a,b, Pietrantonio and Benedict 1999, Mohaghegh et al. 2000). However, these studies evaluating insecticide effects on predators based on topical bioassays or residue assays (as was conducted here) must be supplemented with laboratory studies evaluating sublethal effects and with field studies evaluating community level effects (such as intraguild predation and predator prey relationships) to determine the impact these products have on predators in agroecosystems.

The susceptibility of pentatomid, geocorid, and nabid predators to both the broadspectrum and selective insecticides evaluated in the laboratory in the current study was lower than expected, and these data combined with the results from the field studies suggest that even when pyrethroid or organophosphorous insecticides are used, predator reestablishment in the field may be very rapid because of tolerance to insecticidal residues on foliar surfaces. Although we did not observe any obvious avoidance of treated surfaces in the laboratory bioassays, it is not possible to determine how large of an effect behavioral avoidance of treated surfaces may have played in the field results.

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