Susceptibilities of *Leptinotarsa decemlineata* (Say) in the North Xinjiang Uygur Autonomous Region in China to Two Biopesticides and Three Conventional Insecticides[1]

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ABSTRACT The Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) in the northern part of the Xinjiang Uygur Autonomous Region has evolved resistance to several pyrethroid and carbamate insecticides. Biological control methods should be a major component of integrated pest management for *L. decemlineata*. Spinosad and abamectin are two biopesticides that have unique mechanisms of action. In this study, the contact toxicities of spinosad and abamectin to *L. decemlineata* fourth instars and adults were determined by topical applications to several field populations. The average LD_{50} values of spinosad and abamectin for adults were 0.1275 and 0.0101 μg (a.i.) per individual, and for fourth instars they were 0.0181 and 0.0016 μg (a.i.) per individual, respectively. These data were among the lowest LD_{50}s ever estimated, which affirmed that the two biopesticides are useful for *L. decemlineata* control in north Xinjiang. Susceptibilities to the two biopesticides varied slightly but significantly among tested field populations and the variations did not result from cross-resistance to conventional insecticides. Regarding stomach toxicities, the LC_{50} values of spinosad applied to excised potato leaves for second instars, third instars, fourth instars, and adults were 0.2840, 0.4093, 1.2413, and 2.3783 mg/L (a.i.), respectively. The LC_{50} values of abamectin for second instars, third instars, fourth instars, and adults were 0.0036, 0.0088, 0.0177, and 0.2591 mg/L (a.i.), respectively. The two biopesticides were most toxic to second instars, followed by third, then fourth instars, and they were least toxic to adults. These data suggested that the appropriate timing for spinosad or abamectin spraying is to early larval stages.

KEY WORDS *Leptinotarsa decemlineata*, spinosad, abamectin, biopesticide, toxicity

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The Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is a notorious defoliator of potato, *Solanum tuberosum* L. (Solanaceae). By its voracious feeding, the beetle also devastates other solanaceous crops such as eggplant, tomato, pepper, and tobacco (Osman 2009,

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2010). This beetle invaded China in the 1990s from Kazakhstan. Since then, it has spread eastward, and currently it is distributed through most of northern portion of the Xinjiang Uygur Autonomous Region where it often causes high yield losses of potatoes (Jiang et al. 2010, 2011).

The control of this pest over the last 50 years has relied on the use of insecticides throughout the world (Alyokhin et al. 2008, Alyokhin 2009), and northern Xinjiang is no exception. After the initial outbreak of *L. decemlineata*, growers typically applied systemic formulations of carbofuran as an in-furrow application at planting or as a seed treatment to control this pest. A few years later after a considerable decrease in carbofuran efficiency, three pyrethroids, cyhalothrin, deltamethrin, and α-cypermethrin, became the major chemicals used to control *L. decemlineata*. To a lesser extent, organophosphates and endosulfan also were used. The indiscriminate use of insecticides without knowledge of effective management strategies has resulted in widespread control failures and rapid development of resistance to all major classes of insecticides (Alyokhin 2009, Mohamadi et al. 2010, Zichová et al. 2010). Currently, Colorado potato beetles in northern Xinjiang have developed resistance to several classes of insecticides, particularly pyrethroids such as cyhalothrin and deltamethrin, and carbamates such as carbofuran and carbosulfan (Jiang et al. 2010, 2011).

Biological control should be a major component of integrated pest management for *L. decemlineata* control (Lacey et al. 1999). Using biopesticides has proven to be an efficient means of control in potato pest management programs, because their use reduces pollution and delays the development of resistance to other classical insecticides (Barčič et al. 2006). Spinosad and abamectin are biopesticides produced through the fermentation of *Saccharopolyspora spinosa* and *Streptomyces avermitilis*, respectively. Spinosad is a mixture of spinosyns A and D, and abamectin is comprised of a mixture of avermectin B_{1a} and B_{1b} (Thompson et al. 2000, Gouamene-Lamine et al. 2003, Kirst 2010). The spinosyns are activators of nicotinic acetylcholine receptors in insects, and they also disrupt transmission in some GABA-ergic neurons in insects, which may contribute to their insecticidal activity (Thompson et al. 2000, Kirst 2010). On the other hand, the mechanisms of action for abamectin are involved in γ-aminobutyric acid- and glutamate-gated Cl^- channel (Fritz et al. 1979, Kass et al. 1980, Wolstenholme & Rogers 2005, Moreno et al. 2010, Crump & Omura 2011). Spinosad (Scott 1998, Sparks et al. 1998, McLeod et al. 2002, Huang et al. 2004, Kirst 2010) and abamectin (Putter et al. 1981, Lasota & Dybas 1991) can effectively control many pest species of Lepidoptera, Diptera, Coleoptera, Homoptera, and Orthoptera. These biopesticides generally show less activity against many beneficial predators, as well as mammals and other aquatic and avian animals (Sarfraz et al. 2005, Kirst 2010). These two biopesticides should be excellent selections for the efficient control of *L. decemlineata* in northern Xinjiang.

The objectives of this study were to perform laboratory bioassays to establish baselines for abamectin and spinosad for comparisons to currently available insecticides, for future resistance monitoring, for testing their efficacy against different developmental stages of *L. decemlineata*, and for appropriate application timing.

The pharmacokinetics of abamectin and its metabolites have been elucidated. Three major oxidative metabolites, namely 24-hydroxymethyl, 26-hydroxymethyl, and 3″-O-desmethyl abamectin have been identified (Zeng et al. 1996).

The same metabolic route is followed in *L. decemlineata*, which has resulted in the resistance to abamectin in some strains in the United States (Argentine & Clark 1990, Argentine et al. 1992, Yoon et al. 2002, Gouamene-Lamine et al. 2003). Considerable variation in susceptibility to spinosad was found among *L. decemlineata* populations never exposed to this insecticide (Tolman et al. 2005). Therefore, we also investigated whether these two biopesticides showed any cross resistance to conventional insecticides. This might provide some insight into how resistance may occur and how these products should be used to minimize the risks of resistance.

Materials and Methods

Insects and insecticides. Potato is a single-season crop grown from May to August or September in the Xinjiang Uygur Autonomous Region in China. Overwintered adult Colorado potato beetles emerge and feed on the leaves of potato plants in early to mid-May. Overwintered beetles were collected from potato fields in the cities of Urumqi (43.71°N, 87.39°E), Changji (43.87°N, 87.25°E), and Tacheng (46.45°N, 82.59°E), and from the counties of Qitai (44.03°N, 89.56°E), Qapqal (43.81°N, 81.20°E), Nilka (43.79°N, 82.50°E), and Tekes (43.23°N, 81.82°E). Sampling locations were chosen at random in the study areas. The beetles were reared in an insectary at 28 ± 1°C, under a 16 h:8 h (light:dark) photoperiod, and 50–60% relative humidity using fresh potato foliage as food. Insects used in these experiments were first-generation larvae of different instars and adults at least seven days after emergence from pupae.

The technical-grade active ingredients used in bioassays were cyhalothrin (95% a.i.; Nanjing Red Sun Group Limited Company, Jiangsu, China), carbosulfan (90% a.i.; Nanjing Red Sun Group Limited Company), azinphos-methyl (98.9% a.i.; Sigma-Aldrich Shanghai Trading Co Ltd., Shanghai, China), spinosad (90% a.i.; Dow AgroSciences Company, Indianapolis, IN), and abamectin (95% a.i.; Nanjing Baofeng Insecticide Factory, Jiangsu, China). The two commercial formulations evaluated were spinosad (Success®, SC, 2.5% wt:vol, Dow AgroSciences Company) and abamectin (EC, 2.0% wt:vol, Nanjing Red Sun Group Limited Company).

Topical bioassay. A topical application was used to assess the contact toxicity of the test materials on *L. decemlineata* fourth instars and adults. Insecticides were dissolved individually in analytical-grade acetone. Based on preliminary assays, more than five concentrations within a mortality range of 0–100% were used. Ten larvae were treated individually with 0.22 μL of insecticide solution, applied to the dorsal abdominal segment with a 10-μL microsyringe connected to a microapplicator (Hamilton Company, Reno, NV). Similarly, 10 adults were treated individually with 1.1 μL of insecticide solution applied to the ventral area of the abdomen with a 50-μL microsyringe. Each control larva or adult received 0.22 or 1.1 μL of acetone, respectively. Control mortality was typically less than 10%. Three to five replications per concentration were performed.

After treatment, the test insects were placed in Petri dishes (9 cm diameter and 1.5 cm height) containing fresh potato leaves and kept under environmental conditions outlined for beetle rearing.

Ingestion bioassay. A leaf-dip method was adopted for the ingestion bioassay to evaluate the stomach toxicity of these materials for larvae and adults from Tekes potato fields. Based on preliminary assays, five to nine concentrations within a mortality range of approximately 0–100% were prepared from serial dilutions with distilled water. Fresh potato leaves were individually dipped into one of the solutions for 5 seconds, removed, and dried for 2 hours under airflow on filter paper. The treated leaves were then placed in Petri dishes (9 cm diameter and 1.5 cm height). The beetles were starved for at least 4 hours prior to the experiment. Then, ten larvae or adults were transferred to each dish. Distilled water was applied as a blank control. All treatments were replicated three to five times. After treatments, the Petri dishes were kept under environmental conditions outlined for beetle rearing.

Spinosad was a slower-acting compound and death was delayed for several days (Mota-Sanchez et al. 2006). Consequently, the mortalities were assessed 10 days after treatment. Mortalities for abamectin were assessed five days after treatment. For cyhalothrin, carbosulfan, and azinphosmethyl, mortalities were assessed two days after treatment. The surviving larvae were transferred to untreated fresh leaves every other day. The treated larvae were considered dead if they could not move their legs and body after one leg was touched with a fine needle, and the treated adults were considered dead if they were unable to right themselves or walk a distance equal to their own body length when disturbed with a fine needle (Sharif et al. 2007).

Data analysis. Abbott's formula (Abbott 1925) was used to correct the data for control mortality. In general, the mortality of an organism under various concentrations of an insecticide is binomial. Probit analysis is used to transform the sigmoid dose-response curve to a straight line, and then to calculate doses needed to cause 50% mortality (LD_{50} or LC_{50}), their fiducial limits, and the slope of the line relating probit mortality to the log dose using POLO Plus logit probit software (LeOra Software Company, Petaluma, CA, USA). For the ingestion bioassay, the exact amount of ingested material was not measured. We used the known concentrations (a.i.) to evaluate LC_{50}s. Resistance ratios (RRs) were determined by comparing LD_{50} values of every population with corresponding LD_{50} values of the Tekes field population, which has been confirmed susceptible to tested conventional insecticides (Jiang et al. 2010, 2011). Insecticide resistance level was classified by using RRs on the basis of the standard of Torres-Vila et al. (2002), where susceptibility (RR = 1.0), low-resistance (RR = 2.1–10.0), moderate resistance (RR = 10.1–30.0), high resistance (RR = 30.1–100.0), and very high resistance (RR > 100.0) were determined. The differences between LD_{50} values were compared by their non-overlapping fiducial limits.

Results

Analyses of resistance ratios among different field strains. The LD_{50} values of cyhalothrin, carbosulfan, and aziphosmethyl were determined by topical applications in 2010. Resistance levels (LD_{50}) for several field populations were compared with the Tekes field population from 2009 (Jiang et al. 2010, 2011) (Table 1).

For cyhalothrin, the adults from Nilka, Qapqal, Tacheng, and Changji exhibited high to very high levels of resistance, with the resistance ratios of

38.7, 77.7, 616.9, and 384.2, respectively; the adults from Tekes and Qitai were still susceptible. Moreover, the fourth instars from Nilka, Qapqal, and Changji showed very high levels of resistance, with the resistance ratios of 169.8, 924.6, and 5868.0, respectively; the larvae from Tekes, Urumqi, and Qitai remained susceptible (Table 1).

For carbosulfan, the adults from all four tested field populations (Tekes, Qapqal, Changji, and Qitai) were still susceptible. However, the fourth instars from Nilka, Qapqal, Changji, Urumqi, and Qitai showed low to high levels of resistance (Table 1).

For azinphosmethyl, the adults from all three tested field populations and the fourth instars from all six field populations were still susceptible (Table 1).

Susceptibilities of spinosad and abamectin by topical application. Several field populations representing different resistance levels to both cyhalothrin and carbosulfan were selected from northern Xinjiang Uygur Autonomous Region. The susceptibilities of these field populations to spinosad and abamectin by topical application were investigated to determine contact toxicities.

Fourth instars were more sensitive than adults to both spinosad and abamectin. For spinosad, the average LD_{50} value for the adults was 0.1275 μg (a.i.) per individual, and for the fourth instars the average LD_{50} value was 0.0181 μg (a.i.) per individual. The larvae were 7.0-fold more sensitive than the adults. For abamectin, the average LD_{50} value for adults was 0.0101 μg (a.i.) per individual and for the fourth instars it was 0.0016 μg (a.i.) per individual. The larvae were 6.4-fold more sensitive than the adults (Table 2).

Based on the non-overlapping fiducial limits of LD_{50} values, slight but significantly different susceptibilities were found among tested field populations. For spinosad, the relative susceptible indices ranged 0.9–1.4 for adult populations and 0.7–3.0 for fourth instar populations. For abamectin, the relative susceptible index ranged 0.1–1.0 for adult populations and 0.3–2.7 for fourth instar populations (Table 2).

Susceptibilities of spinosad and abamectin determined by the leaf dip method. The susceptibilities of adults and second, third, and fourth instars of *L. decemlineata* from Tekes were evaluated by the leaf-dip method to determine stomach toxicities. Spinosad and abamectin were most toxic to second instars, followed by third and fourth instars, and least toxic to adults. The relative toxicity indices to second, third, and fourth instars and adults for spinosad and abamectin were 8.4, 5.8, 1.9, and 1.0, and 72.0, 29.4, 14.6, and 1.0, respectively (Table 3). Second instars were most sensitive to abamectin and spinosad.

Discussion

Our previous results (Jiang et al. 2010, 2011) showed that the adults and the fourth instars of some field populations of *L. decemlineata* had developed resistance to cyhalothrin, deltamethrin, α-cypermethrin, carbofuran, and carbosulfan. Because the resistance in *L. decemlineata* may vary markedly from region to region, from field to field, and between adults and larvae, depending on the patterns of insecticide use and pesticide pressure (Silcox et al. 1985, Zehnder 1986, Zehnder & Gelernter 1989, Pourmirza 2005), we expanded our monitoring

Table 1. Toxicity of several conventional insecticides in some field strains of *L. decemlineata* adults and fourth instars.

Insecticide	Stage	Strain	N	X2	df	Slope (±SE)	LD_{50}(µg(a.i.)/ individual)(95%FL)	RR[a]
Cyhalothrin	Adult	Tekes	300	4.2	9	1.823 (±0.19)	0.0021 (0.0011–0.0032)	1.3
		Nilka	268	4.0	8	1.150 (±0.05)	0.0619 (0.0359–1.1066)	38.7
		Qapqal	272	4.1	8	1.214 (±0.07)	0.1243 (0.0786–0.1969)	77.7
		Tachenghang	257	3.2	8	1.133 (±0.07)	0.9871 (0.3584–2.7184)	616.9
		Changji	270	3.7	8	1.340 (±0.08)	0.6147 (0.4215–0.8791)	384.2
		Qitai	240	2.4	7	1.956 (±0.12)	0.0066 (0.0045–0.0097)	4.1
	4th-instar	Tekes	210	3.4	6	1.931 (±0.15)	0.0005 (0.0001–0.1951)	1.0
		Nilka	240	4.1	7	2.134 (±0.26)	0.0849 (0.0671–0.1074)	169.8
		Qapqal	270	4.5	8	1.245 (±0.22)	0.4623 (0.2351–0.9090)	924.6
		Changji	300	5.0	9	1.858 (±0.30)	2.9340 (2.1847–3.9404)	5868.0
		Urumqi	210	3.8	6	1.201 (±0.13)	0.0001 (0.0001–0.0021)	0.2
		Qitai	210	3.8	6	1.336 (±0.10)	0.0001 (0.0001–0.0022)	0.2
Carbosulfan	Adult	Tekes	270	3.2	8	2.824 (±0.19)	0.4678 (0.3728–0.5871)	0.5
		Qapqal	269	4.0	8	3.514 (±0.12)	0.5071 (0.4197–0.6126)	0.5
		Changji	270	2.7	8	2.898 (±0.62)	0.6371 (0.5097–0.8342)	0.7
		Qitai	240	1.9	7	2.663 (±0.08)	0.1371 (0.1145–0.1642)	0.1
	4th-instar	Tekes	210	3.1	6	2.315 (±0.21)	0.0151 (0.0063–0.0228)	1.3
		Nilka	240	2.5	7	4.437 (±0.37)	0.3356 (0.2909–0.3871)	30.0
		Qapqal	240	3.8	7	3.045 (±0.35)	0.1814 (0.1430–0.2302)	16.2
		Changji	210	3.0	6	3.184 (±0.29)	0.2749 (0.2345–0.3222)	24.5
		Urumqi	210	2.4	6	6.445 (±0.52)	0.3491 (0.3244–0.3756)	31.1
		Qitai	210	3.5	6	2.663 (±0.20)	0.1371 (0.1145–0.1642)	12.2
Azinphosmethyl	Adult	Tekes	271	2.9	8	2.236 (±0.22)	3.7216 (2.9127–4.8413)	1.0
		Qapqal	270	4.3	8	1.718 (±0.17)	2.1323 (1.5314–2.9690)	0.6
		Changji	270	3.4	8	3.047 (±0.14)	0.8861 (0.7047–1.1142)	0.2

Table 1. Continued.

Insecticide	Stage	Strain	N	X2	df	Slope (±SE)	LD_{50}(µg(a.i.)/ individual)(95%FL)	RR[a]
	4th-instar	Tekes	300	3.7	9	1.926 (±0.18)	0.3828 (0.1479–0.5478)	1.1
		Nilka	240	0.9	7	2.499 (±0.23)	1.3434 (1.0773–1.6753)	3.9
		Qapqal	240	3.4	7	1.877 (±0.27)	0.7725 (0.6373–1.1375)	2.2
		Changji	210	2.8	6	2.502 (±0.33)	0.7958 (0.6209–1.0201)	2.3
		Urumqi	210	3.4	6	2.324 (±0.29)	0.9217 (0.7825–1.1306)	2.6
		Qitai	210	3.7	6	1.935 (±0.26)	0.2415 (0.1898–0.2929)	0.7

[a] RR = Resistance Ratio; determined by comparing LD_{50} values of each test populations with those of the Tekes reference population.

Table 2. Susceptibilities of several field populations of *L. decemlineata* fourth instars or adults in the Xinjiang Uygur Autonomous Region to topically applied spinosad and abamectin.

Stage	Strain	Insecticide	N	X^2	df	Slope (±SE)	LD_{50}(μg(a.i.)/individual)(95%FL)	RSI[a]
Adult	Tekes	Spinosad	270	3.6	8	1.832 (±0.22)	0.1112 (0.0827–0.1496)	1.0
	Qapqa l	Spinosad	360	6.0	11	1.923 (±0.18)	0.1000 (0.0688–0.1452)	0.9
	Changji	Spinosad	240	2.4	7	3.003 (±0.32)	0.1378 (0.1121–0.1693)	1.2
	Urumqi 2	Spinosad	240	3.1	7	1.623 (±0.25)	0.1608 (0.1158–0.2232)	1.4
	Tekes	Abamectin	240	3.5	7	1.993 (±0.09)	0.0164 (0.0072–0.0267)	1.0
	Qapqal 1	Abamectin	240	2.8	7	1.387 (±0.08)	0.0013 (0.0008–0.0021)	0.1
	Qapqal 2	Abamectin	360	5.7	11	2.203 (±0.21)	0.0070 (0.0050–0.0090)	0.4
	Changji	Abamectin	240	2.4	7	1.435 (±0.14)	0.0131 (0.0091–0.0153)	0.8
	Urumqi 2	Abamectin	240	3.2	7	1.097 (±0.10)	0.0127 (0.0073–0.0223)	0.8
4th-instar	Tekes	Spinosad	240	3.4	7	1.985 (±0.15)	0.0104 (0.0072–0.0149)	1.0
	Nilka	Spinosad	300	4.2	9	1.478 (±0.20)	0.0076 (0.0055–0.0105)	0.7
	Qapqal	Spinosad	240	2.6	7	1.971 (±0.26)	0.0310 (0.0243–0.0396)	3.0
	Changji	Spinosad	210	2.8	6	2.413 (±0.30)	0.0300 (0.0230–0.0391)	2.9
	Urumqi 1	Spinosad	210	3.3	6	2.009 (±0.09)	0.0221 (0.0167–0.0292)	2.1
	Urumqi 2	Spinosad	243	3.8	7	3.310 (±0.43)	0.0176 (0.0150–0.0240)	1.7
	Qitai	Spinosad	210	3.1	6	2.033 (±0.26)	0.0079 (0.0058–0.0108)	0.8
	Tekes 1	Abamectin	240	3.4	6	1.604 (±0.30)	0.0012 (0.0003–0.0053)	1.0
	Tekes 2	Abamectin	300	4.5	9	1.663 (±0.24)	0.0032 (0.0023–0.0044)	2.7
	Nilka 1	Abamectin	240	2.1	7	1.286 (±0.32)	0.0018 (0.0004–0.0084)	1.5
	Nilka 2	Abamectin	270	3.6	8	1.690 (±0.17)	0.0014 (0.0010–0.0020)	1.2
	Changji	Abamectin	180	1.3	5	1.539 (±0.11)	0.0003 (0.0001–0.0006)	0.3

[a] RSI = Relative Susceptibility Index; determined by comparing the LD_{50} value of each population for spinosad with the corresponding LD_{50} value of the Tekes reference population.

Table 3. Susceptibilities to spinosad in *L. decemlineata* adults and larvae (second, third, and fourth instars) as determined by the leaf-dip method.

Insecticide	Stage	N	X^2	Df	Slope (±SE)	LC50 (mg/L(a.i.) (95% FL)	RTI[a]
Spinosad	Adult	268	3.9	8	1.972 (±0.11)	2.3783 (1.8054–3.1328)	1.0
	2nd-instar	240	2.8	7	2.660 (±0.32)	0.2840 (0.2294–0.3515)	8.4
	3rd-instar	267	3.4	8	2.705 (±0.28)	0.4093 (0.3441–0.4869)	5.8
	4th-instar	241	2.5	7	1.402 (±0.18)	1.2413 (0.8732–1.7647)	1.9
Abamectin	Adult	239	2.3	7	1.129 (±0.08)	0.2591 (0.1529–0.4390)	1.0
	2nd-instar	270	2.8	8	2.111 (±0.25)	0.0036 (0.0025–0.0053)	72.0
	3rd-instar	263	4.2	8	2.623 (±0.32)	0.0088 (0.0066–0.0117)	29.4
	4th-instar	241	2.0	7	1.728 (±0.16)	0.0177 (0.0131–0.0237)	14.6

[a] Relative Toxicity Index; determined by comparing the LC_{50} value of adults for spinosad with the corresponding LC_{50} value for each larval instar.

to gather data in 2010. The data in the present paper demonstrated that the adults from Nilka, Qapqal, Tacheng, and Changji, and fourth instars from Nilka, Qapqal, and Changji exhibited high to very high levels of resistance to cyhalothrin. Moreover, the fourth instars from Nilka, Qapqal, Changji, Urumqi, and Qitai among six tested field populations showed low to high levels of resistance to carbosulfan. Based on our results, it is apparent that the continued heavy reliance on carbamates and pyrethroids would be problematic in northern Xinjiang. To efficiently control *L. decemlineata* and successfully manage insecticide resistance, novel insecticides with different modes of action should be used alternately.

In the present study, we found that the contact toxicities of spinosad and abamectin to fourth instars and adults were among the lowest ever estimated using the same topical bioassay (Jiang et al. 2010, 2011). Consistent with our results, spinosad was reported to be highly toxic to a susceptible strain in the USA, with an LD_{50} value of 0.348 μg (a.i.) per adult (Mota-Sanchez et al. 2006). Moreover, for a susceptible laboratory strain in the United States, the LD_{50} value of abamectin was found to be from 0.00068 to 0.00110 μg (a.i.) per fourth instar, and 0.00570 and 0.00540 μg (a.i.) per female and male adult, respectively (Yoon et al. 2002, Gouamene-Lamine et al. 2003). These reports and the data from present study affirmed that these two biopesticides are useful for *L. decemlineata* control.

Our topical bioassay revealed that fourth instars were more sensitive than adults to spinosad and abamectin. Because it had been reported that the two biopesticides were toxic by both contact and ingestion (Azimi et al. 2009, Kowalska 2010), a leaf-dip method was adopted for an ingestion bioassay to evaluate stomach toxicities. These experiments showed that the two biopesticides were most toxic to second instars, followed by third and fourth instars, and they were least toxic to adults. It had been reported that LD_{50} values for abamectin for a susceptible laboratory strain from the USA were 0.00003, 0.00014, 0.00100, 0.00110, and 0.00560 μg (a.i.) per individual for first, second, third, and fourth instars, and adults, and relative toxicity indices were 186.7, 40.0, 5.6, 5.1, and 1.0, respectively (Gouamene-Lamine et al. 2003). In an independent experiment (Osman 2010) at the concentration of 7.2 μg (a.i.) per ml, three days after treatment abamectin killed 88.9%, 73.3%, 71.1%, and 48.9% of first, second, third, and fourth instars, respectively. Using the same bioassay at the concentration of 60 μg (a.i.) per ml, three days after treatment spinosad killed 86.7%, 84.4%, 73.3%, and 57.8% of first, second, third, and fourth instars, respectively (Osman 2010). These results indicate that neonates are the most sensitive developmental stage to abamectin and spinosad. Therefore, for optimal results, the two biopesticides should be applied to the early larval-stage of the beetles.

It has been reported that a higher level of cytochrome P450 monooxygenase activity resulted in an enhanced *in vivo* and *in vitro* oxidative metabolism to abamectin, which was responsible for resistance to abamectin in a *L. decemlineata*-resistant strain (Yoon et al. 2002, Gouamene-Lamine et al. 2003). Our previous results using three enzyme inhibitors (triphenyl phosphate, diethylmeleate, and piperonyl butoxide) showed that cytochrome P450 monooxygenases and esterases may be involved in insecticide resistance in some field populations (Jiang et al. 2010, 2011). Thus, we can pose the question, do the higher levels of P450 monooxygenase and esterase activities in resistant field populations lead to any potential cross resistance to the two biopesticides? In the present paper, we found that the susceptibilities of the fourth instars and the adults to the two biopesticides varied slightly but significantly among several field populations. However, the susceptible differences to the two biopesticides were not positively correlated with the resistance ratio to cyhalothrin or carbofuran, indicating that the susceptible differences did not result from cross-resistance to conventional insecticides. Similar susceptible differences were found among *L. decemlineata* populations never exposed to the two insecticides in Canada (Tolman et al. 2005). The occurrence of low but significant levels of resistance against the two biopesticides in field-collected populations before the first field application indicates that the risk of resistance evolution exists.

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