Evidence of Field-Evolved Resistance to Organophosphates and Pyrethroids in *Chrysoperla carnea* (Neuroptera: Chrysopidae)

Author(s): Attaullah Khan Pathan, Ali H. Sayyed, Muhammad Aslam, Muhammad Razaq, Ghulam Jilani, and Mushtaq Ahmad Saleem
Source: Journal of Economic Entomology, 101(5):1676-1684. 2008.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/0022-0493(2008)101[1676:EOFRTO]2.0.CO;2
URL: http://www.bioone.org/doi/full/10.1603/0022-0493%282008%29101%5B1676%3AEOFRTO%5D2.0.CO%3B2

Evidence of Field-Evolved Resistance to Organophosphates and Pyrethroids in *Chrysoperla carnea* (Neuroptera: Chrysopidae)

ATTAULLAH KHAN PATHAN,[1] ALI H. SAYYED,[2,3] MUHAMMAD ASLAM,[4] MUHAMMAD RAZAQ,[5] GHULAM JILANI,[6] AND MUSHTAQ AHMAD SALEEM[5]

J. Econ. Entomol. 101(5): 1676–1684 (2008)

ABSTRACT The toxicity of some of the most commonly used insecticides in the organophosphate and pyrethroid classes were investigated against different *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) populations collected over three consecutive years (2005–2007). The populations were tested using leaf dip bioassays for residual effects and topical applications to measure the response of larvae that would come into direct contact with field application of insecticides. In leaf dip assays, the LC_{50} (micrograms per milliliter; 120 h) values for chlorpyrifos and profenofos were in the range of 59.3–1,023 and 180.02–1,118 respectively. The LC_{50} values for lambda-cyhalthrin, alphamethrin, and deltamethrin were 359.08–2,677, 112.9–923.5, and 47.81–407.03, respectively. The toxicity for the above insecticides in topical application was similar to toxicity in leaf dip assays. The susceptibility of a laboratory population, which was locally developed and designated as (Lab-PK), to deltamethrin was comparable with another susceptible laboratory population. Resistance ratios for five field populations were generally low to medium for deltamethrin, but high to very high for chlorpyrifos, profenofos, lambda-cyhalthrin and alphamethrin compared with the Lab-PK population. Our data also suggested that the five field populations had multiple resistance to two classes of insecticides. The populations showed resistance to two organophosphates tested and to lambda-cyhalthrin and alphamethrin; however, resistance to deltamethrin was only found at two locations. This pattern indicates occurrence of two divergent patterns of resistance within pyrethroids. The resistance to the insecticides was stable across 3 yr, suggesting field selection for general fitness had also taken place in various populations of *C. carnea*. The broad spectrum of resistance and stability of resistance to insecticides in *C. carnea* in the current study suggested that it could be a prime candidate for mass releases and compatible with most spray programs.

KEY WORDS *Chrysoperla carnea*, organophosphates, pyrethroids, resistance, stability of resistance

Insect natural enemies can develop resistance to insecticides in the field just as their hosts can, although they are perceived to be slow to develop pesticides resistance in either the laboratory or the field because of a combination of biological, ecological, and biochemical (lower detoxification capacity) factors (Roush et al. 1990). Insecticides also can affect the evolution of insecticide resistance in natural enemies by direct exposure to spray in the field, or indirectly by consumption insecticide-treated hosts (Wu et al. 2004, Wu and Miyata 2005). Some predators or parasitoids have developed resistance in the field, and they can survive field rates of insecticides (Roush et al. 1990, Isman et al. 1997, Toews and Subramanyam 2004). For example, predatory phytoseiid mites have developed a high level of resistance, and they can survive insecticide applications in the field (Headley and Hoy 1987, Whitten and Hoy 1999).

Insect pests cause up to 56% losses of crops in Pakistan, and 20–40% of these losses are in cotton, *Gossypium hirsutum* L. (Ahmed 1999). Insecticides with broad toxicity to pests and their natural enemies are widely used in cotton insect pest management. The overuse of insecticides can lead to the elimination of natural enemies and give rise to phenomena such as pest resurgence, occurrence of secondary pests, and selection of populations of resistant insects (Ahmed 1995). The integrated pest management (IPM) concept proposed by Stern et al. (1959) advocates both chemical and biological control in agricultural systems. However, biological control agents are difficult to maintain when pesticides are applied to control key pests because natural enemies are often more sensitive to insecticides compared with the pests. To maintain natural enemies in IPM systems, predators and para-

[1] IPM Sub-Station–PARC, University College of Agriculture, B.Z. University, Multan, Pakistan-60800.
[2] Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, United Kingdom.
[3] Corresponding author, e-mail: h.a.sayyed@sussex.ac.uk.
[4] University College of Agriculture and Environmental Sciences, The Islamya University of Bahawalpur, Bahawalpur, Pakistan.
[5] Department of Entomology, University College of Agriculture, B. Z. University, Multan, Pakistan 60800.
[6] Institute of Plant and Environmental Protection, National Agricultural Research Institute, Park Road Islamabad, Pakistan.

0022-0493/08/1676–1684$04.00/0

sitoids should be resistant or tolerant to several groups of pesticides.

The larvae of *Chrysoperla* spp. are among the most efficient predators of many important agricultural insect pests (Lingren et al. 1968), particularly *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), which is the most abundant species in the genus (Van den Bosh and Hagen 1966). They can inhabit many diverse agroecosystems, and they are easily mass reared (Rajakulendran and Plapp 1982). Interest in using beneficial predators as a component of IPM programs, for field and horticultural crops, has recently increased as growers seek alternatives to insecticides for managing insect pests. Some *Chrysoperla* species are known to exhibit tolerance or resistance to insecticides, which also makes the predator compatible with most IPM systems (Pree et al. 1989). However, generalizations about the tolerance of *C. carnea* to pesticides that are based on tests with single colonies or single populations may be inappropriate (Roush et al. 1990).

The use of pesticide-resistant natural enemies in agroecosystems might prevent pest resurgences and secondary pest outbreaks in many crops in which chemical control of pests is practiced. Knowledge of the evolution of resistance to insecticides in natural enemies in the field could enable the development of IPM programs that minimize the use of insecticides (Landis et al. 2000). Natural enemies are a key component of IPM, and they are often recommended as the first line of defense in an IPM program (Lugojja et al. 2001). A survey of growers in Pakistan in 2005 showed that 13, 27, and 57% of cotton growers questioned used organophosphate, pyrethroid, and neonicotinoid insecticides, respectively (average spray interval was 5 d; A.K.P. and M.A., unpublished data). In the current study, we were interested to establish whether *C. carnea* had developed resistance to conventional insecticides in the field and whether the resistance levels were sufficiently high enough to allow a resistant strain to be used in an IPM system in which field rates of insecticides were used.

Materials and Methods

Insects. Adults *C. carnea* were collected from central Pakistan (Dera Ismail Khan, Dera Ghazi Khan, Multan, Bahawalpur, and Rahimyar Khan districts) from 2005 to 2007 (Table 1). Adults were collected as described previously (Cohen 1989) with an insect sampling device (John W. Hock Company, Gainesville, FL) or ventilated plastic vials. Collections were made either at 0800–1100 hours in summer or at 1100–1500 hours in winter. The collected adults were kept in plastic jars (12 by12 by 20 cm) with artificial diet (yeast, honey, and distilled water; 1:2:4 ratios). The adults were transported to the laboratory of IPM Station PARC, University College of Agriculture, B. Z. University, Multan. The laboratory susceptible strain of *C. carnea* was collected from Multan in 1999, and it was designated as Lab-PK. This population was reared in the laboratory for >60 generations without exposure to insecticides. In the laboratory adults were kept at 25 ± 1°C, 60 ± 5% RH, and a photoperiod of 18:6 (L:D) h. in a plastic rearing cage (23 by 38 by 38 cm) with ventilation holes on both sides. Black glossy paper was hung in the cage for egg laying. The eggs were placed in petri dishes, and hatched larvae were fed on eggs of *Sitotroga cerealella* Oliver.

Insecticides. The formulated insecticides used were Curacron EC (profenofos 500 g/liter, Sygenta Crop Protection, Basil, Switzerland), Lorsban EC (chlorpyrifos 400 g/liter, Dow Agro Sciences, Hitchin, United Kingdom), Karate EC (lambda-cyhalthrin 25 g/liter, Syngenta Limited, Jealot Hill, United Kingdom), Bestox EC (alphamethrin 50 g/liter, FMC, Philadelphia, PA), and Decis Super EC (deltamethrin 100 g/liter, Bayer Crop Sciences, Montpellier, France).

Bioassays. *Residual Effect.* Bioassays were conducted on 2- to 3-d-old larvae of *C. carnea* from field-collected populations by using a standard dip bioassay method as recommended by the insecticide resistance action committee (Sayyed et al. 2000). The test solutions of insecticides were freshly prepared in distilled water. Filter papers (Whatman no. 41, 90 mm in diameter; Whatman, Maidstone, United Kingdom) were either dipped in test solutions or in distilled water for controls. The filter papers were placed on the middle plat of test chambers made of three plates (10 by 7.5 cm), with the middle plate covered with muslin cloth to confine larvae on this plate, and all three plates were clumped together. Each insecticide was tested within a range of five to none concentrations to determine the LC_{50} value, including controls, and each concentration was replicated three times. Twelve larvae were placed in the middle plate in each replication, and the total number of larvae tested per concentration was 36. The larvae were provided with eggs of *S. cereallela* for food. The bioassays were kept at a temperature 25 ± 2°C, 65% RH, and a photoperiod of 14:10 (L:D) h. Mortality was assessed after 120-h exposure to insecticides.

Topical Application. This experiment was conducted to measure the response of larvae that had direct contact with insecticides as could occur in the field. Larvae were directly treated with insecticide by using an auto-microapplicator (Burkard Manufacturing Co. Ltd., Hertfordshire, England) equipped with a 1-ml glass syringe; 0.5 μl of an insecticide solution in water was applied to individual larva. The treated larvae were kept as described above. Each insecticide was tested with six concentrations to determine the LC_{50} value, and each concentration was replicated three times. Each insecticide concentration was applied to 30 larvae, and 30 control larvae were treated with water only. One larva was placed in each well of a 24-well plate, and 10 larvae were used per replication. Mortality was assessed after 120-h exposure to insecticides.

Stability of Resistance. A decline or increase in resistance to organophosphates and pyrethroids in populations found in a field from 1 yr to the next was measured by calculating a R (respond per month) value. The R value was estimated as follows:

$$R = [\log (\text{final } LC_{50}) - \log (\text{initial } LC_{50}) / n$$

Table 1. Residual effects of organophosphates and pyrethroids to laboratory susceptible and field populations of *C. carnea*

Insecticide	Location	Yr	LC_{50} ppm (95% FL)	Slope ± SEM	χ^2	df	*P*	RR[a]	DR[b]	n^c
Chlorpyrifos	Lab-PK		6.17 (4.26–8.94)	2.11 ± 0.38	1.12	3	0.77	1		180
	D.I. Khan	Nov. 2005	138.47 (103.25–185.70)	2.14 ± 0.34	0.78	3	0.85	22		180
		Oct. 2006	132.90 (97.80–180.59)	2.10 ± 0.34	1.06	3	0.79	22	−0.001	180
		Oct. 2007	68.15 (1.56–2,973.33)	1.34 ± 0.73	5.05	4	0.28	11	−0.02	216
	D.G. Khan	Dec. 2005	810.02 (649.98–1,009.47)	3.8 ± 0.79	1.01	3	0.80	132		180
		Dec. 2006	863.60 (697.50–1,069.25)	3.68 ± 0.74	1.83	3	0.61	140	0.002	180
		Nov. 2007	1,023 (315–3,323)	3.46 ± 1.57	5.9	3	0.12	166	0.006	180
	Multan	Dec. 2005	110.07 (75.59–160.29)	1.91 ± 0.33	1.01	3	0.80	18		180
		Dec. 2006	64.79 (42.41–98.99)	1.48 ± 0.24	1.24	4	0.87	11	−0.02	180
		Nov. 2007	58.29 (38.78–87.63)	1.63 ± 0.25	1.38	3	0.71	9	−0.004	180
	Bahawalpur	Dec. 2005	435.89 (328.45–578.46)	2.67 ± 0.50	4.45	3	0.22	71		180
		Dec. 2006	409.10 (307.72–543.86)	2.81 ± 0.54	0.97	3	0.81	66	−0.002	180
		Nov. 2007	328.02 (245.99–437.41)	2.00 ± 0.31	1.97	3	0.58	53	−0.008	180
	R.Y. Khan	Dec. 2005	215.10 (155.57–297.39)	2.65 ± 0.51	1.51	3	0.68	34		180
		Dec. 2006	238.18 (173.93–326.18)	2.20 ± 0.37	0.93	3	0.82	39	0.004	180
		Nov. 2007	265.80 (195.61–361.17)	2.10 ± 0.34	1.06	3	0.79	43	0.004	180
Profenofos	Lab-PK		16.21 (12.19–21.56)	2.43 ± 0.44	2.22	3	0.52	1		180
	D.I. Khan	Nov. 2005	504.95 (420.50–606.35)	4.02 ± 0.75	0.13	3	0.99	31		180
		Oct. 2006	490.70 (387.51–621.38)	3.13 ± 0.64	1.30	3	0.73	30	−0.001	180
		Oct. 2007	542.32 (449.40–654.45)	3.71 ± 0.63	0.55	3	0.91	33	0.004	180
	D.G. Khan	Dec. 2005	749.56 (608.33–923.57)	2.79 ± 0.43	2.78	3	0.43	46		180
		Dec. 2006	968.04 (398.05–2,354.22)	3.37 ± 1.34	4.00	3	0.26	60	0.01	180
		Nov. 2007	1,118 (868–1,441)	2.62 ± 0.57	1.49	3	0.68	69	0.005	180
	Multan	Dec. 2005	589.78 (485.74–716.10)	3.40 ± 0.55	2.23	3	0.53	36		180
		Dec. 2006	585.02 (499.48–785.21)	1.48 ± 0.24	2.56	3	0.46	36	0.0003	180
		Nov. 2007	673.95 (555.43–817.76)	3.20 ± 0.49	0.76	3	0.85	42	0.005	180
	Bahawalpur	Dec. 2005	425.13 (329.60–548.33)	3.28 ± 0.65	0.06	3	0.99	26		180
		Dec. 2006	497.25 (386.83–639.20)	2.87 ± 0.51	2.14	3	0.54	31	0.006	180
		Nov. 2007	518.63 (390.69–688.46)	2.45 ± 0.44	4.35	3	0.23	32	0.002	180
	R.Y. Khan	Dec. 2005	180.02 (133.37–242.98)	2.65 ± 0.51	0.51	3	0.92	11		180
		Dec. 2006	216.24 (163.80–285.47)	2.48 ± 0.44	1.19	3	0.75	13	0.007	180
		Nov. 2007	256.21 (174.72–375.71)	1.58 ± 0.35	4.64	3	0.20	16	0.006	180
Lambda-cyhalthrin	Lab-PK		23.60 (17.00–32.75)	2.24 ± 0.39	1.39	3	0.71	1		180
	D.I. Khan	Nov. 2005	660.00 (367.26–1,186.09)	0.94 ± 0.50	1.21	3	0.75	29		180
		Oct. 2006	1,193.0 (464–3,069)	0.84 ± 0.50	0.15	3	0.99	52	0.02	180
		Oct. 2007	948.18 (518.05–1,735.43)	1.07 ± 0.50	0.03	3	0.99	41	−0.008	180
	D.G. Khan	Dec. 2005	1,197.0 (973–1,474)	2.89 ± 0.57	0.58	3	0.90	52		180
		Dec. 2006	1,812.0 (1,292–2,542)	1.88 ± 0.53	0.32	3	0.96	79	0.02	180
		Nov. 2007	2,677 (1,989–3,603)	2.10 ± 0.53	1.85	3	0.60	113	0.02	180
	Multan	Dec. 2005	1,322.0 (1,038–1,685)	2.39 ± 0.54	0.19	3	0.98	57		180
		Dec. 2006	1,824.0 (1,389–2,394)	2.37 ± 0.55	0.09	3	0.99	79	0.01	180
		Nov. 2007	2,324 (1,803–2,996)	3.11 ± 0.66	0.3	3	0.96	101	0.009	180
	Bahawalpur	Dec. 2005	404.50 (311.76–524.82)	2.82 ± 0.61	0.18	3	0.98	18		180
		Dec. 2006	537.04 (418.43–689.19)	2.43 ± 0.55	0.66	3	0.88	25	0.01	180
		Nov. 2007	701.19 (533.82–921.02)	2.10 ± 0.53	0.57	3	0.90	30	0.01	180
	R.Y. Khan	Dec. 2005	359.08 (255.28–505.08)	2.38 ± 0.59	0.05	3	0.99	16		180
		Dec. 2006	403.35 (280.33–580.36)	2.00 ± 0.54	0.19	3	0.98	17	0.004	180
		Nov. 2007	502.41 (351.82–717.45)	1.73 ± 0.52	0.15	3	0.98	22	0.008	180
Alphamethrin	Lab-PK		10.55 (8.08–13.79)	2.53 ± 0.44	1.87	3	0.60	1		180
	D.I. Khan	Nov. 2005	174.45 (131.96–230.63)	2.36 ± 0.42	0.38	3	0.94	17		180
		Oct. 2006	196.81 (141.98–272.81)	1.85 ± 0.37	0.18	3	0.98	19	0.004	180
		Oct. 2007	238.35 (171.44–331.38)	1.66 ± 0.35	0.2	3	0.97	23	0.007	180
	D.G. Khan	Dec. 2005	418.16 (331.46–527.53)	2.60 ± 0.55	0.66	3	0.88	40		180
		Dec. 2006	701.39 (522.59–941.38)	2.21 ± 0.54	0.03	3	0.99	66	0.02	180
		Nov. 2007	923.50 (651.45–1,309.15)	2.34 ± 0.58	0.59	3	0.90	88	0.01	180
	Multan	Dec. 2005	112.92 (25.84–493.48)	2.49 ± 1.13	5.82	3	0.12	11		180
		Dec. 2006	152.41 (127.27–182.53)	3.42 ± 0.60	0.81	3	0.84	14	0.01	180
		Nov. 2007	184.36 (155.13–219.10)	3.68 ± 0.62	0.65	3	0.88	17	0.007	180
	Bahawalpur	Dec. 2005	270.03 (199.29–365.89)	2.71 ± 0.62	0.08	3	0.99	10		180
		Dec. 2006	299.64 (220.88–406.48)	2.44 ± 0.58	0.46	3	0.93	28	0.004	180
		Nov. 2007	374.97 (269.07–522.55)	1.88 ± 0.53	0.32	3	0.96	36	0.008	180
	R.Y. Khan	Dec. 2005	283.59 (226.18–355.57)	2.38 ± 0.38	0.71	3	0.87	27		180
		Dec. 2006	294.73 (189.05–459.48)	1.69 ± 0.53	0.39	3	0.94	28	0.001	180
		Nov. 2007	351.44 (226.56–545.13)	1.48 ± 0.51	0.87	3	0.83	33	0.006	180
Deltamethrin	Lab-PK		14.33 (10.73–19.14)	2.23 ± 0.40	4.03	3	0.25	1		180
	D.I. Khan	Nov. 2005	64.25 (49.11–84.05)	2.26 ± 0.39	0.99	3	0.80	4		180

Continued on following page

Table 1. Continued

Insecticide	Location	Yr	LC_{50} ppm (95% FL)	Slope ± SEM	χ^2	df	*P*	RR[a]	DR[b]	*n*[c]
		Oct. 2006	47.81 (30.04–76.11)	1.54 ± 0.36	0.1	3	0.99	3	−0.01	180
		Oct. 2007	77.98 (55.88–108.81)	1.62 ± 0.34	0.1	3	0.99	5	0.02	180
	D.G. Khan	Dec. 2005	200.91 (152.70–264.34)	2.62 ± 0.59	0.36	3	0.94	14		180
		Dec. 2006	262.91 (202.45–341.42)	2.30 ± 0.54	2.64	3	0.45	18	0.01	180
		Nov. 2007	407.03 (300.11–552.05)	2.00 ± 0.53	0.19	3	0.98	28	0.02	180
	Multan	Dec. 2005	198.17 (152.84–256.94)	2.36 ± 0.40	0.11	3	0.99	14		180
		Dec. 2006	231.93 (184.74–291.18)	2.53 ± 0.40	0.01	3	1.00	16	0.006	180
		Nov. 2007	185.70 (146.05–236.11)	2.67 ± 0.45	0.18	3	0.98	13	−0.008	180
	Bahawalpur	Dec. 2005	270.03 (199.29–365.89)	2.71 ± 0.62	0.18	3	0.98	10		180
		Dec. 2006	133.64 (99.11–180.19)	1.98 ± 0.53	0.57	3	0.90	9	−0.02	180
		Nov. 2007	131.78 (95.67–181.52)	1.85 ± 0.52	0.27	3	0.97	9	0	180
	R.Y. Khan	Dec. 2005	51.16 (38.42–68.12)	2.44 ± 0.44	1.2	3	0.75	4		180
		Dec. 2006	51.11 (37.12–70.36)	2.18 ± 0.41	0.39	3	0.94	4	0	180
		Nov. 2007	54.24 (40.20–73.19)	2.23 ± 0.41	3.37	3	0.34	4	0.002	180

[a] Resistance ratio = LC_{50} field population ÷ LC_{50} of susceptible strain.
[b] Rate of increase or decrease in resistance.
[c] Number of larvae tested in a bioassay.

where n is the number of months after which a second population was collected from the same field. Increase or decrease in resistance is reflected in positive and negative values of R (Attique et al. 2006).

Data Analysis. Mortality data were corrected by Abbott's formula (Abbott 1925) where necessary, and the estimates of LC_{50} values and their 95% fiducial limits (FLs) were obtained by probit analysis (Finney 1971) by using the software POLO-PC (LeOra 2003). Because of the inherent variability of bioassays, pairwise comparisons of LC_{50} values were made at the 1% significance level to determine whether the 95% FLs for two treatments did not overlap (Litchfield and Wilcoxon 1949). Resistance ratios were determined by dividing the LC_{50} values of field populations with the LC_{50} value of the Lab-PK population. The slopes of regression lines were compared using *t*-test in GLIM (Crawley 1993).

Insecticide resistance level was classified by using resistance ratios (RRs) in terms widely accepted as follows: susceptibility (RR = 1), tolerance to low-resistance (RR = 2–10), moderate resistance (RR = 11–50), high resistance (RR = 51–100), and very high resistance (RR > 100) (Ahmad et al. 2007).

Results

Toxicity of Test Chemicals to Susceptible Population. The results of residual bioassays with the Lab-PK population (Table 1) showed that the chlorpyrifos was significantly ($P < 0.01$) more toxic than profenofos, lambda-cyhalthrin, and deltamethrin. Profenofos, alphamethrin, and deltamethrin had similar toxicity to the Lab-PK population. Lambda-cyhalthrin was the least toxic insecticide tested. The slopes of regression lines of chlorpyrifos, profenofos, lambda-cyhalthrin, alphamethrin, and deltamethrin were similar ($P > 0.05$).

The toxicity of profenofos, lambda-cyhalthrin, alphamethrin and deltamethrin in topical bioassays was similar however chlorpyrifos was significantly more toxic than lambda-cyhalthrin, alphamethrin and deltamethrin (Table 2; non overlapping of 95% FL).

Toxicity of Insecticides to Field Populations in Residual Bioassays. The toxicity of all five insecticides was significantly lower for field populations ($P < 0.01$) compared with Lab-PK (95% FL did not overlap; Table 1). The levels of resistance to chlorpyrifos in samples from all five locations were generally high, with resistance ratios 9- to 166-fold. Of 15 field populations tested with chlorpyrifos in 3 yr, six populations showed high to very high levels of resistance (53- to 166-fold). The highest level of resistance (166-fold) was observed in 2007 from D.G. Khan, whereas the lowest (9-fold) was from Multan (Table 1).

Of 15 populations tested for profenofos, two populations had high levels of resistance (60- to 69-fold) compared with the Lab-PK population, and the remaining 13 populations had moderate levels of resistance (11- to 46-fold). The lowest level of resistance was found in populations from R.Y. Khan in all 3 yr, whereas the highest levels were found in populations from D.G. Khan in 2006 and 2007 (Table 1). The slopes of the regression lines were significantly steeper ($P < 0.05$) than the Lab-PK population for three populations from D.I. Khan, one from D.G. Khan, two from Multan, and one population from Bahawalpur.

Among the 15 populations tested with lambda-cyhalthrin, seven populations showed high to very high levels of resistance (52- to 113-fold), and the remaining eight populations showed moderate levels of resistance (16- to 41-fold) compared with the Lab-PK population. The lowest level of resistance was observed from R.Y. Khan in 2005, 2006, and 2007 with resistance of 16-, 17-, and 22-fold, respectively. The slopes of the regression lines for the majority of field populations were similar to the Lab-PK population except populations collected in 2005 and 2006 from D.I. Khan, which were significantly shallower ($P < 0.05$) than the slope of the Lab-PK population (Table 1).

Only two populations from D.G. Khan collected in 2006 and 2007 showed high levels of resistance to alphamethrin with resistance of 66- and 88-fold, respectively, compared with the Lab-PK population. The remaining 13 populations had moderate levels of

Table 2. Toxicity of organophosphates and pyrethroids in topical bioassays to laboratory susceptible and field populations of *C. carnea*

Insecticide	Location	Yr	LC_{50} (95% FL)	Slope ± SE	χ^2	df	*P*	RR[a]	DR[b]	n^c
Chlorpyrifos	Lab-PK		6.13 (2.92–12.89)	1.58 ± 0.47	1.3	3	0.73	1		150
	D.I. Khan	Oct. 2006	233.25 (152.83–355.99)	2.63 ± 0.73	0.60	3	0.90	38		150
		March 2007	277.39 (188.95–407.23)	2.58 ± 0.68	1.10	3	0.78	45	0.006	150
		Dec. 2007	196.43 (116.01–332.60)	2.40 ± 0.74	0.98	3	0.81	32	−0.017	150
	D.G. Khan	Oct. 2006	611.72 (449.93–831.69)	3.15 ± 0.76	0.30	3	0.96	100		150
		March 2007	723.93 (509.39–1,028.83)	2.50 ± 0.62	2.29	3	0.51	118	0.006	150
		Dec. 2007	619.52 (432.94–886.51)	2.62 ± 0.66	1.10	3	0.78	101	−0.007	150
	Multan	Oct. 2006	322.75 (218.70–476.30)	2.34 ± 0.61	0.15	3	0.99	53		150
		March 2007	252.33 (161.66–393.85)	2.35 ± 0.66)	1.11	3	0.77	41	−0.009	150
		Dec. 2007	145.72 (88.13–240.96)	1.87 ± 0.47	1.41	3	0.70	24	−0.03	150
	Bahawalpur	Oct. 2006	350.86 (228.79–538.06)	2.03 ± 0.57	0.44	3	0.93	57		150
		March 2007	283.98 (185.56–434.58)	2.28 ± 0.62	0.23	3	0.98	46	−0.008	150
		Dec. 2007	299.54 (189.07–474.55)	2.03 ± 0.49	0.98	3	0.81	49	0.003	150
	R.Y. Khan	Oct. 2006	538.93 (404.03–718.86)	3.63 ± 1.01	0.16	3	0.98	88		150
		March 2007	458.22 (309.45–678.49)	2.91 ± 0.96	0.98	3	0.81	75	−0.006	150
		Dec. 2007	313.90 (205.31–479.91)	2.16 ± 0.59	0.90	3	0.82	51	−0.018	150
Profenofos	Lab-PK		14.9 (9.19–24.0)	2.37 ± 0.69	0.66	3	0.88	1		150
	D.I. Khan	Oct. 2006	637.84 (455.82–892.54)	2.87 ± 0.71	0.91	3	0.82	43		150
		March 2007	658.76 (498.52–870.52)	3.53 ± 0.83	0.78	3	0.85	44	0.001	150
		Dec. 2007	557.17 (375.79–826.08)	2.61 ± 0.70	0.76	3	0.86	38	−0.008	150
	D.G. Khan	Oct. 2006	1,027.0 (695.0–1,518.0)	2.81 ± 0.93	0.19	3	0.98	69		150
		March 2007	1,027.0 (695.0–1,518.0)	2.81 ± 0.93	0.19	3	0.98	69	0	150
		Dec. 2007	878.63 (541.42–1,425.88)	2.59 ± 0.94	1.21.	3	0.75	59	−0.007	150
	Multan	Oct. 2006	847.48 (621.70–1,155.25)	4.31 ± 1.42	0.11	3	0.99	57		150
		March 2007	711.75 (516.69–980.44)	2.88 ± 0.69	0.78	3	0.85	48	−0.006	150
		Dec. 2007	605.93 (429.32–855.36)	2.87 ± 0.72	0.47	3	0.92	41	−0.007	150
	Bahawalpur	Oct. 2006	730.22 (555.30–960.22)	3.48 ± 0.79	0.26	3	0.97	49		150
		March 2007	629.51 (475.28–833.80)	3.57 ± 0.86	0.26	3	0.97	42	−0.005	150
		Dec. 2007	684.68 (506.52–925.52)	3.15 ± 0.74	0.52	3	0.91	46	0.004	150
	R.Y. Khan	Oct. 2006	279.10 (200.92–387.72)	2.86 ± 0.69	0.82	3	0.84	19		150
		March 2007	293.60 (213.14–404.43)	2.88 ± 0.69	0.78	3	0.85	20	0.002	150
		Dec. 2007	237.29 (166.54–338.10)	2.87 ± 0.74	0.40	3	0.94	16	−0.01	150
Lambda-cyhalthrin	Lab-PK		24.81 (14.73–41.78)	2.12 ± 0.56	0.62	3	0.89	1		150
	D.I. Khan	Oct. 2006	1,129.0 (853.0–1,494.0)	3.43 ± 0.96	1.69	3	0.64	46		150
		March 2007	1059.0 (725.0–1,548.0)	2.50 ± 0.85	0.38	3	0.94	43	−0.002	150
		Dec. 2007	787.21 (358.68–1,727.70)	1.14 ± 0.78	1.21	3	0.75	32	−0.014	150
	D.G. Khan	Oct. 2006	1036.0 (663.0–1,619.0)	2.12 ± 0.83	1.01	3	0.80	42		150
		March 2007	1000.0 (680.0–1,470.0)	2.54 ± 0.86	0.91	3	0.82	40	−0.001	150
		Dec. 2007	1276.0 (832.0–1,956.0)	2.07 ± 0.82	1.39	3	0.71	51	0.012	150
	Multan	Oct. 2006	1205.0 (868.0–1,672.0)	2.82 ± 0.87	0.61	3	0.89	49		150
		March 2007	1092.0 (817.0–1,459.0)	3.37 ± 0.94	0.64	3	0.89	44	−0.004	150
		Dec. 2007	2404.0 (1481.0–3,901.0)	2.59 ± 0.94	1.03	3	0.79	97	0.038	150
	Bahawalpur	Oct. 2006	570.96 (410.78–793.62)	2.85 ± 0.88	0.15	3	0.99	23		150
		March 2007	579.46 (446.46–752.09)	3.79 ± 0.98	0.30	3	0.96	23	0.0005	150
		Dec. 2007	517.96 (331.31–809.75)	2.12 ± 0.83	1.31	3	0.73	21	−0.005	150
	R.Y. Khan	Oct. 2006	570.96 (410.78–793.62)	2.85 ± 0.88	0.15	3	0.99	23		150
		March 2007	682.82 (445.38–1,046.85)	2.07 ± 0.82	1.53	3	0.67	28	0.006	150
		Dec. 2007	553.58 (357.67–856.80)	2.10 ± 0.82	1.21	3	0.75	22	−0.01	150
Alphamethrin	Lab-PK		9.72 (6.37–14.83)	2.63 ± 0.73	0.60	3	0.90	1		150
	D.I. Khan	Oct. 2006	304.32 (186.36–496.94)	2.32 ± 0.88	0.20	3	0.98	31		150
		March 2007	274.57 (169.19–445.59)	2.59 ± 0.94	1.34	3	0.72	28	−0.004	150
		Dec. 2007	233.44 (138.11–394.56)	1.63 ± 0.54	0.16	3	0.98	24	−0.008	150
	D.G. Khan	Oct. 2006	609.89 (362.16–1,027.07)	1.76 ± 0.88	0.15	3	0.99	63		150
		March 2007	583.26 (399.94–850.62)	2.45 ± 0.84	0.12	3	0.99	60	−0.002	150
		Dec. 2007	767.87 (429.17–1,373.88)	1.84 ± 0.82	0.11	3	0.99	79	0.013	150
	Multan	Oct. 2006	390.46 (250.89–607.66)	2.15 ± 0.83	1.53	3	0.67	40		150
		March 2007	338.13 (229.60–497.96)	2.73 ± 0.90	0.71	3	0.87	35	−0.005	150
		Dec. 2007	356.67 (243.19–523.09)	2.65 ± 0.88	1.53	3	0.67	37	0.003	150
	Bahawalpur	Oct. 2006	378.81 (257.73–556.77)	2.54 ± 0.86	1.04	3	0.79	39		150
		March 2007	386.31 (278.10–536.61)	3.00 ± 0.91	1.05	3	0.79	40	0.001	150
		Dec. 2007	323.19 (200.47–521.04)	2.27 ± 0.86	0.98	3	0.81	33	−0.008	150
	R.Y. Khan	Oct. 2006	410.21 (294.43–571.54)	2.88 ± 0.89	1.44	3	0.70	42		150
		March 2007	357.96 (241.92–529.65)	2.58 ± 0.88	1.05	3	0.79	37	−0.005	150
		Dec. 2007	321.09 (217.29–474.47)	2.81 ± 0.93	0.19	3	0.98	33	−0.005	150
Deltamethrin	Lab-PK		13.46 (8.62–21.01)	2.35 ± 0.66	1.11	3	0.77	1		150
	D.I. Khan	Oct. 2006	97.78 (60.96–156.83)	2.40 ± 0.89	1.08	3	0.78	7		150
		March 2007	106.55 (77.71–146.10)	3.44 ± 1.01	2.29	3	0.51	8	0.003	150

Continued on following page

Table 2. Continued

Insecticide	Location	Yr	LC_{50} (95% FL)	Slope ± SE	χ^2	df	*P*	RR[a]	DR[b]	n^c
		Dec. 2007	90.80 (49.11–167.88)	1.96 ± 0.85	1.24	3	0.74	7	−0.008	150
	D.G. Khan	Oct. 2006	277.67 (207.72–371.18)	3.31 ± 0.92	1.39	3	0.71	21		150
		March 2007	247.24 (177.99–343.43)	3.00 ± 0.91	1.05	3	0.79	18	−0.004	150
		Dec. 2007	310.19 (231.55–415.55)	3.22 ± 0.91	0.26	3	0.97	23	0.011	150
	Multan	Oct. 2006	138.42 (99.58–192.39)	2.85 ± 0.88	0.15	3	0.98	10		150
		March 2007	155.10 (115.77–207.77)	3.22 ± 0.91	0.26	3	0.97	12	0.004	150
		Dec. 2007	171.55 (124.86–241.22)	2.80 ± 0.87	0.37	3	0.95	13	0.005	150
	Bahawalpur	Oct. 2006	111.93 (80.39–155.84)	3.15 ± 0.95	1.05	3	0.78	8		150
		March 2007	97.75 (66.02–144.75)	2.91 ± 0.96	1.37	3	0.71	7	−0.005	150
		Dec. 2007	85.01 (45.0–160.57)	2.01 ± 0.87	1.06	3	0.79	6	−0.007	150
	R.Y. Khan	Oct. 2006	70.10 (48.96–100.36)	2.54 ± 0.64	0.40	3	0.94	5		150
		March 2007	73.0 (50.24–106.08)	2.38 ± 0.61	1.01	3	0.79	5	−0.005	150
		Dec. 2007	55.72 (37.58–82.61)	2.61 ± 0.70	0.76	3	0.86	4	−0.013	150

[a] Resistance ratio = LC_{50} field population ÷ LC_{50} of susceptible strain.
[b] Rate of increase or decrease in resistance.
[c] Number of larvae tested in a bioassays including controls.

resistance ranging from 11- to 40-fold compared with the Lab-PK population (Table 1). The slopes of the regression lines for the majority of field populations were similar to that for the Lab-PK population. However, two populations collected from Multan in 2006 and 2007 showed significantly steeper slopes ($P < 0.05$) than the slope of the Lab-PK population (Table 1).

Test populations were comparatively less tolerant to deltamethrin than they were to the other pyrethroids (lambda-cyhalthrin and alphamethrin). The populations collected from D.G. Khan and Multan had low levels of resistance, whereas populations from D.I. Khan, Bahalwalpur, and R.Y. Khan were merely tolerant to deltamethrin. However, the LC_{50} values of the populations were significantly greater than the LC_{50} value for the Lab-PK population (Table 1). The slopes of the regression lines for all 15 field populations were similar to the slope for the Lab-PK population (Table 1).

Toxicity of Insecticides to Field Populations in Topical Bioassays. Similar to the residual bioassays, the toxicity of the insecticides in the topical assays was lower than their toxicity to the Lab-PK population (Table 2). For example, resistance to organophosphates (chlorpyrifos and profenofos) was moderate to very high, ranging from 16- to 118-fold compared with the Lab-PK population. The highest resistance levels were found in populations collected from D.G. Khan in all 3 yr, and the lowest levels of resistance to profenofos were found in populations from R.Y. Khan (Table 2).

Resistance to lambda-cyhalthrin and alphamethrin was moderate to high in the field populations, ranging from 22- to 97-fold compared with the Lab-PK population. However, a moderate level of resistance to deltamethrin was only found in populations from D.G. Khan and Multan, with resistance of 10- to 23-fold. The populations collected from D.I. Khan, Bahawalpur, and R.Y. Khan showed a tolerant to low level of resistance to deltamethrin (Table 2).

Stability of Resistance across 3 Yr. From 2005 to 2007, the resistance to chlorpyrifos and profenofos remained the same. There was no significant change in the rate of increase or decrease in resistance to the insecticides (Tables 1 and 2). Resistance to lambda-cyhalthrin, alphamethrin, and deltamethrin increased significantly in 2007 over levels found in 2005 in populations collected from D.G. Khan (Table 1). However, there was no indication of significant change in the resistance when the same populations were tested in topical bioassays and resistance was similar across the 3 yr (Table 2).

Discussion

Present studies were carried out to evaluate the toxicity of two organophosphate and three pyrethroid insecticides to *C. carnea* collected from central Pakistan. The studies were conducted for three consecutive years (2005–2007), and the bioassay results showed varying degrees of resistance in field populations. Resistance to chlorpyrifos, profenofos, lambda-cyhalothrin, alphamethrin, and deltamethrin was generally high. It has been suggested that insects should not be considered resistant until a resistance ratio of 10 is exhibited (Torres-Vila et al. 2002). Accordingly, we considered <10-fold resistance ratios we found with deltamethrin in both residual and topical assays to be tolerance rather than resistance. Studies of resistant populations depends on comparisons with standard susceptible laboratory strains and these can be hypersensitive to some compounds (Gonzalez-Cabrera et al. 2001). The susceptibility of our Lab-PK population to deltamethrin was significantly lower than was found for another susceptible population of *C. carnea* (Vineland) from Ontario, Canada, used by Pree et al. (1989). However, the field populations that we compared with our Lab-PK population still showed a significantly high level of field-evolved resistance. In contrast to our study, some carbamate and pyrethroids had been shown to be toxic to eggs and larvae of *C. externa*, with 100% mortality in laboratory assays (Schneider et al. 2006).

Tolerance or resistance to some old-generation pyrethroids and organophosphates has been reported

previously (Pree et al. 1989); however, to the best of our knowledge, there are no recent reports of field-evolved resistance in *C. carnea.* Our study shows that the five field populations have multiple resistance to two classes of insecticides. For example, all five locations are resistant to two organophosphates tested and to the pyrethroids lambda-cyalthrin and alphamethrin; however, resistance to deltamethrin is only found from two locations (Table 2). The data suggest occurrence of two divergent patterns of resistance within pyrethroids, indicating more than one mechanism of resistance exist for imparting resistance to organophosphate and pyrethroids in *C. carnea.* In Pakistan, it is a common practice to tank mix insecticides to control cotton pests, but it would be premature to conclude that cross-resistance existed between these compounds in *C. carnea.* Further studies are therefore necessary to confirm whether the cross-resistance between various insecticides exists by selecting a *C. carnea* population in the laboratory with representative pyrethroids and organophosphates. Pyrethroid resistance can be due to modifications to the target site of these insecticides or due to enhanced activity of detoxifying enzymes (Cohen and Morin 1988, Pree et al. 1989). Similarly the predominant mechanism of resistance to organophosphate also could also be due to enhanced activity of detoxifying enzymes (Gunning et al. 1998) or a modification of the enzyme acetylcholinesterase, which is the target site of organophosphate and carbamate insecticides (Hama 1983). However, further studies are required to confirm the involvement of the enzymes in resistance to organophosphates or pyrethroids in *C. carnea.* Previously, Pree et al. (1989) showed that resistance to organophosphates and pyrethroids was associated with monooxygenases and esterases in field populations of *C. carnea* from southern Ontario, Canada. Similarly, Ishaaya and Casida (1981) and Grafton-Cardwell and Hoy (1986) found the presence of an esterase-mediated detoxification route for pyrethroids in *C. carnea.*

Comparison among resistance ratios as measured by topical and leaf dip assays indicated a similar pattern of resistance to all insecticides tested. Our studies confirmed the study by Pree et al. (1989) that used topical method only to measure resistance to old-generation pyrethroids and organophosphates in *C. carnea.* Most of the standard insecticide bioassays are capable of detecting resistance. However, selection of the appropriate exposure method on certain life stages can often improve discrimination between susceptible and resistant genotypes. The current study indicated that both topical and residual methods provided a sensitive measure of resistance in larvae of *C. carnae.* The toxicity of insecticides in topical bioassays was lower than the residual bioassays, which was unexpected. We were expecting toxicity to be higher in topical bioassays because the amount of insecticide penetrating would be higher than the residual bioassays. The most probably reason for this could be an alternative mechanism of resistance in the field populations. Our data also confirm the involvement of multiple resistance in the populations tested. We do not have direct evidence to confirm the involvement of lack of cuticle penetration as a mechanism of resistance, but further studies, which are in progress in our laboratory, could provide evidence for various mechanism of resistance to insecticide in *C. carnea.*

The stability of resistance in the absence of exposure to insecticides is very important for the utility of natural enemies in IPM. In the current study, rate of decrease of resistance to insecticides in the field populations of *C. carnae* was minimal, which suggests resistance was stable in the populations collected from various locations. The stability of resistance in field populations has at least one explanation. The resistance in the field populations may have been near fixation, leading to a very slow increase in heterozygosity due to combination of biological, ecological, and/or biochemical (lower detoxification capacity) reasons (Roush et al. 1990). Crow (1957) suggested that if resistance alleles conferred negative fitness, then reversion to susceptibility might be quite rapid in the absence of selection. If the development of resistance was accompanied by selection for general fitness (via fitness modifiers), then resistance might persist for longer periods without any selection pressure. It seems that field selection for general fitness may have taken place in the various populations of *C. carnea* we tested. Although we did not measure fitness costs in the current study, the stability of resistance in the field populations we tested suggested that the populations were fitter even in the presence of resistance gene(s). Previously, a decline in resistance to pyrethroids and organophosphates in *C. carnea* was reported to be associated with fitness costs (Jones et al. 1978, Pree et al. 1989). There are diverging opinions on the impact of fitness costs on the reversal of resistance. Tabashnik et al. (1994) suggested that fitness costs caused directly by resistance alleles had an important effect in the field, but Roush (1997) considered that even strong fitness costs had a minimal impact.

Natural enemies are a core component of IPM (Hajek 2004), and application of insecticides is often difficult to avoid in several IPM systems. Therefore, a major challenge is how to maximize the role of natural enemies. The use of pesticide-resistant natural enemies in agroecosystems can, in theory, prevent pest resurgences and secondary pest outbreaks in many crops in which chemical control of pests is practiced. The broad spectrum of resistance and stability of resistance to insecticides in *C. carnea* found in the present studies suggested that it could be compatible with most spray programs and a prime candidate for mass release. For example, if *C. carnea* was used for control of sucking pests (aphids, mites, and whiteflies), the spray schedule for refugia in *Bacillus thuringiensis* (Bt)-transgenic cotton crop systems would not be disrupted and resurgence of plant-sucking pests could be prevented in Bt cotton.

Acknowledgments

A.K.P. is grateful to Muhammad Juman, Asif Sajjad, Afsheen, Laila Khalid, and Tamseela for technical assistance.

The study is funded by Agriculture Linkage Program-Pakistan Agricultural Research Council (ALP-PARC) and is gratefully acknowledged. A.K.P. is also grateful to Waseem Gilani and Juned-ul-Rehman for advise and guidance during the studies. We especially thank Balasubramani Venkatasamy and Paul Johnston for reading earlier version of the manuscript. A.H.S. was supported by Biotechnology and Biological Sciences Research Council project BB/C504927/1.

References Cited

Abbott, S. W. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Ahmad, M., M. I. Arif, and M. Ahmad. 2007. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot. 26: 809–817.

Ahmed, M. 1995. Pest problem of cotton and their management, pp. 67–70. *In* Proceedings National Seminar on Strategies for Increasing Cotton Production, 26–27 April 1995, Punjab Government, Lahore, Pakistan.

Ahmed, Z. 1999. Pest problems of cotton–a regional perspective, pp. 5–20. *In* Proceedings ICAC-CCRI Multan Regional Consultation Insecticide Resistant Management in Cotton, 28 June-1 July 1999. Central Cotton Research Institute, Multan, Pakistan.

Attique, M.N.R., A. Khaliq, and A. H. Sayyed. 2006. Could resistance to insecticides in *Plutella xylostella* (Lep., Plutellidae) be overcome by insecticide mixtures? J. Appl. Entomol. 130: 122–127.

Cohen, A. C. 1989. Ingestion efficiency and protein consumption by a heteropteran predator. Ann. Entomol. Soc. Am. 82: 495–499.

Cohen, A. C., and J. G. Morin. 1988. Species-specific bioluminescent mating displays and morphological relationships of Caribbean species of *Vargula* (Myodocopida). Am. Zool. 28: A109–A109.

Crawley, M. J. 1993. GLIM for ecologists. Blackwell, London, United Kingdom.

Crow, J. F. 1957. Genetics of insect resistance to chemicals. Annu. Rev. Entomol. 2: 227–246.

Finney, D. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge, United Kingdom.

Gonzalez-Cabrera, J., S. Herrero, A. H. Sayyed, B. Escriche, Y. B. Liu, S. K. Meyer, D. J. Wright, B. E. Tabashnik, and J. Ferre. 2001. Variation in susceptibility to *Bacillus thuringiensis* toxins among unselected strains of *Plutella xylostella.* Appl. Environ. Microbiol. 67: 4610–4613.

Grafton-Cardwell, E. E., and M. A. Hoy. 1986. Genetic improvement of common green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae) selection for carbaryl resistance. Environ. Entomol. 15: 1130–1136.

Gunning, R. V., G. D. Moores, and A. L. Devonshire. 1998. Insensitive acetylcholinesterase and resistance to organophosphates in Australian *Helicoverpa armigera.* Pestic. Biochem. Physiol. 62: 147–151.

Hajek, A. E. 2004. Natural enemies: an introduction to biological control. Cambridge University Press, Cambridge, United Kingdom.

Hama, H. 1983. Resistance to insecticides due to reduced sensitivity of acetylcholinesterase, pp. 299–331. *In* G. P. Georghiou and T. Saito, [eds.], Pest resistance to insecticides. Plenum, New York.

Headley, J. C., and M. A. Hoy. 1987. Benefit-cost-analysis of an integrated mite management program for almonds. J. Econ. Entomol. 80: 555–559.

Ishaaya, I., and J. E. Casida. 1981. Pyrethroid esterase(s) may contribute to natural pyrethroid tolerance of larvae of the common green lacewing (Neuroptera, Chrysopidae). Environ. Entomol. 10: 681–684.

Isman, M. B., P. J. Gunning, and K. M. Spollen. 1997. Tropical timber species as sources of botanical insecticides. *In* P. A. Hedin, R. M. Hollingworth, E. P. Masler, J. Miyamoto, and D. G. Thompson [eds.], Phytochemicals for pest control. Am. Chem. Soc. Symp. Ser. 658: 27–37.

Jones, S. L., R. E. Kinzer, D. L. Bull, J. R. Ables, and R. L. Ridgway. 1978. Deterioration of *Chrysopa carnea* (Neuroptera-Chrysopidae) in mass culture. Ann. Entomol. Soc. Am. 71: 160–162.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45: 175–201.

LeOra, S. 2003. Poloplus, a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA.

Lingren, P. D., R. L. Ridgway, C. B. Cowan, J. W. Davis, and W. C. Watkins. 1968. Biological control of bollworm and tobacco budworm by arthropod predators affected by insecticides. J. Econ. Entomol. 61: 1521–1526.

Litchfield, J. T., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 99: 99–103.

Lugojja, F., M. W. Ogenga-Latigo, and N.E.J.M. Smit. 2001. Impact of defoliation on the agronomic performance of sweetpotato in Uganda. Afr. Crop Sci. J. 9: 103–108.

Pree, D. J., D. E. Archibald, and R. K. Morrison. 1989. Resistance to insecticides in the common green lacewing *Chrysoperla carnea* (Neuroptera, Chrysopidae) in southern Ontario. J. Econ. Entomol. 82: 29–34.

Rajakulendran, S. V., and F. W. Plapp. 1982. Synergism of 5 synthetic pyrethroids by chlordimeform against the tobacco budworm (Lepidoptera, Noctuidae) and a predator, *Chrysopa carnea* (Neuroptera, Chrysopidae). J. Econ. Entomol. 75: 1089–1092.

Roush, R. T. 1997. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? Pestic. Sci. 51: 328–334.

Roush, R. T., C. W. Hoy, D. N. Ferro, and W. M. Tingey. 1990. Insecticide resistance in the Colorado potato beetle (Coleoptera, Chrysomelidae–influence of crop-rotation and insecticide use. J. Econ. Entomol. 83: 315–319.

Sayyed, A. H., R. Haward, S. Herrero, J. Ferre, and D. J. Wright. 2000. Genetic and biochemical approach for characterization of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a field population of the diamondback moth, *Plutella xylostella.* Appl. Environ. Microbiol. 66: 1509–1516.

Schneider, M. I., P. Pineda, and G. Smagghe. 2006. Side effects of conventional and non-conventional insecticides on eggs and larvae of *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) in Argentine. Commun. Agric. Appl. Biol. Sci. 71: 425–427.

Stern, V. M., R. F. Smith, R. van den Bosh, and K. S. Hagen. 1959. The integration of chemical and biological control of the spotted alfalfa aphid. Hilgardia 29: 81–101.

Tabashnik, B. E., N. Finson, F. R. Groeters, W. J. Moar, M. W. Johnson, K. Luo, and M. J. Adang. 1994. Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella.* Proc. Natl. Acad. Sci. U.S.A. 91: 4120–4124.

Toews, M. D., and B. Subramanyam. 2004. Survival of stored-product insect natural enemies in spinosad-treated wheat. J. Econ. Entomol. 97: 1174–1180.

Torres-Vila, L. M., M. C. Rodriguez-Molina, A. Lacasa-Plasencia, and P. Bielza-Lino. 2002. Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamates and organophosphates: the Spanish case. Crop Prot. 21: 1003–1013.

Van den Bosh, R., and K. S. Hagen. 1966. Predacious and parasitic arthropods in California cotton fields. Calif. Agric. Exp. Stn. Bull. 820: 32.

Whitten, M. J., and M. A. Hoy. 1999. Genetic improvement and other genetic considerations for improving the efficacy and success rate of biological control, pp. 271–296. *In* T. S. Bellows and T. W. Fisher [eds.], Handbook of biological control: principles and applications of biological control. Academic, New York.

Wu, G., and T. Miyata. 2005. Susceptibilities to methamidophos and enzymatic characteristics in 18 species of pest insects and their natural enemies in crucifer vegetable crops. Pestic. Biochem. Physiol. 82: 79–93.

Wu, G., S. R. Jiang, and T. Miyata. 2004. Seasonal changes of Methamidophos susceptibility and biochemical properties in *Plutella xylostella* (Lepidoptera: Yponomeutidae) and its parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae). J. Econ. Entomol. 97: 1689–1698.

Received 16 January 2008; accepted 8 April 2008.