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FIELD EVOLVED RESISTANCE AND INHERITANCE PATTERNS OF LABORATORY SELECTED STRAINS OF ORIENTAL ARMYWORM, *Mythimna separata,* AGAINST LAMBDA-CYHALOTHRIN AND ITS CROSS RESISTANCE TO OTHER INSECTICIDES

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This is the first report of resistance development including the mode, inheritance of resistance, and life parameters in field collected as well as lambda-cyhalothrin selected laboratory strains of *Mythimna separata*. Field collected and laboratory selected strains were further assessed for cross-resistance with organophosphate insecticides: chlorpyrifos, triazophos, profenophos, quinalphos, and metha-midophos. For field resistant and susceptible strains, the LC_{50} was determined for 2016 and 2017. In laboratory studies, lambda-cyhalothrin-resistant *M. separata* (Lambda-R) were selected for 14 generations and reciprocally crossed; F_1 progenies were simultaneously backcrossed with susceptible parents (Lambda-S) to examine the phenomena of refuge strategy by instantaneously reducing the resistance developed in *M. separata* resulting in F_2 progenies with diluted resistance against lambda-cyhalothrin. Both field collected (G_1) and laboratory selected populations (G_{14}) (Parents, F_1 and F_2 progenies) were tested for their LC_{50} values. RR ratio, through bioassay, was higher in the lambda-cyhalothrin selected strain than the susceptible strain, which confirms resistance in *M. separata* to lambda-cyhalothrin. The *h* value explicitly reveals that resistance is incomplete dominant and autosomal in nature and is controlled by more than one factor. Reciprocal crosses of F_1 progeny show cross resistance to other organophosphate insecticides, which shows that different insecticides can control the insect. Cross resistance of the lambda-cyhalothrin selected strain further showed that the LC_{50} value of organophosphate insecticides was closer to the field collected population of insects showing higher levels of cross resistance of lambda-cyhalothrin and other organophosphate insecticides sprayed for oriental armyworm. These results are helpful to manage outbreaks of oriental armyworm in maize and cotton fields.
Keywords: *Mythimna separata*, cross resistance, resistance, inheritance.

INTRODUCTION

Oriental armyworm, *Mythimna separata* (Walker) (= *Pseudaletia separata* and *Leucania separata* (https://www.uniprot.org/taxonomy/271217)) is a polyphagous pest caterpillar found in China, Korea, Japan, southeast Asia, and some parts of Russia, Australia and Oceania. Plant hosts on which it feeds include maize, rice, wheat, barley, and rye (http://www.agroatlas.ru/en/content/pests/Mythimna_separata/index.html). In Pakistan the caterpillar is known to attack wheat (http://www.fao.org/3/v9976e/v9976e05.htm) and paddy, thus also known as paddy armyworm in Pakistan (https://www.plantwise.org/knowledgebank/datasheet/45093).

Extensive use of insecticides causes severe selection pressure on insect populations often leading to resistance. Target site and metabolic processes are considered as important to insecticide resistance. Gene flow involving rapid spread after an initial occurrence of the resistant allele is also a major reason for insecticide resistance development (Raymond and Marquine, 1994). This phenomenon is very evident in areas where pesticides are sprayed in higher quantities than areas of low insecticide use (Caprio, 2001). Hence, understanding insecticide resistance development is of key importance prior to the development of new insecticides (Mallet, 1989).

Genetic studies of insecticide resistance can provide better information for developing integrated resistance management (IRM) programs for insect pests. Different major and minor genes interact, which results in various resistance mechanisms and different alleles at a locus during long periods of time (Groeters and Tabashnik, 2000). In order to slow down development of insecticide resistance, a clear understating of the inheritance of resistance is required to

elaborate a better plan (Shi *et al.,* 2011) for resistance mitigation. Using different insecticides at the same time can result in cross resistance, thus reducing the efficacy of insecticides to control insect pests, resulting in less successful integrated resistance management programs (Kranthi *et al.,* 2001). In Pakistan, continued use of pesticides has resulted in resistance development in insects. In Pakistan, insects have already developed resistance against endosulfan, pyrethroids, organophosphates, carbamates, and some new chemistry insecticides (Ahmed *et al.,* 1995, 1997, 1999, 2001, 2003). *Helicoverpa armigera* was found to show low levels of resistance, under laboratory conditions, for chlorpyrifos and profenofos (Ahmad *et al.,* 1995).

Oriental armyworm outbreaks can only be controlled by using pyrethroids and organophosphates insecticides but, the species has been found to show resistance against pyrethroids (Yu, 1987; Yang and Gong, 1992; Yang *et al.,* 1995; Dong *et al.,* 2014). Likewise, resistance development in oriental armyworm, *Mythimna separata*, was found against lambda-cyhalothrin and chlorpyrifos in China (Zhao *et al.,* 2018) and against thia-methoxam (Yaqoob *et al.,* 2018). Because the chance of developing resistance in oriental armyworm due to its migratory behavior, thus limited studies has been conducted with this pest (Yang *et al.,* 1995). However, after frequent outbreaks, there has been a reconsideration of insecticide resistance development in this pest and the observance of its population dynamics in an effort to sort out mechanisms responsible for resistance (Jiang *et al.,* 2014). GST, CarE and cytochrome 450 were found to be responsible for resistance development against thia-methoxam (Yaqoob *et al.,* 2018).

In our experiments, for inheritance of resistance experiments, a lambda-cyhalothrin selected strain was reciprocally crossed with susceptible *M. separata* to understand maternal effects, degree of dominance, and number of genes involved in resistance evolution. Further, F_1 progeny were back-crossed with resistant strains to determine if resistance is sex-linked or autosomal. These genetic crosses were helpful in determining dominance; partial dominanance; recessive, monogenic, or polygenic mode of resistance inheritance of the laboratory selected lambda-cyhalothrin population. Cross resistance of pyrethroid lambda-cyhalothrin to other insecticides including chlorpyrifos, triazphos, profenophos, quinalphos, methamidophos is important to successful Integrated Resistance Management (IRM) programs.

MATERIALS AND METHODS

***Field studies Insect collection and rearing*:** Approximately 2000 *M. separata* larvae (3rd instar) were collected from different fields located in four districts (Faisalabad, Multan, Khanewal and Vehari) of Punjab, Pakistan, where lambda-cyhalothrin was sprayed during 2016 and 2017. After collection, larvae were kept separate, in plastic jars covered with muslin cloth, to maintain homogeneous populations. Cultures were maintained on maize. Temperature was 27±2°C and humidity was 65±5 RH. Caterpillars collected from four districts were further selected with lambda-cyhalothrin and were designated as a resistant strain of lambda-cyhalothrin. A susceptible strain was also collected from the field and was maintained in the laboratory for 14 generations without spray.

Table 1. History of selection to produce lambda-cyhalothrin resistant strains of *Mythimna separata.*

Generation	Concentration µg/ml	No. of larvae exposed	No. of larvae dead	%Mortality
G_1	130	1800	879	48.83
G_2	180	1100	168	15.27
G_3	250	1200	150	12.50
G_4	350	1150	110	9.56
G_5	480	1200	100	8.33
G_6	550	1300	90	6.92
G_7	630	1000	48	4.80
G_8	750	1250	44	3.52
G_9	850	1030	32	3.10
G_{10}	950	1100	17	1.54
G_{11}	1050	1200	10	0.83
G_{12}	1100	1150	6	0.52
G_{13}	1150	1100	4	0.36
G_{14}	1200	1000	3	0.30

***Laboratory insect collection*:** Insects resistant and susceptible to lambda-cyhalothrin were collected from lambda-cyhalothrin sprayed fields at the Research Farm of the University of Agriculture, Faisalabad, Pakistan. Larvae were collected from maize that was in the tasseling stage and leaves and tassels were brought to the laboratory and larvae were taken out and reared on maize. Almost 2000 larvae were collected and reared on maize that had been sprayed with lambda-cyhalothrin. Temperature was 28±1ºC, RH was above 65% and D:L period was kept at 14:10.

***Insecticide formulations*:** Commercially available insecticides including lambda-cyhalothrin (Karate 2.5 EC), Chlorpyrifos (Lorsban 40EC), triazophos (Triazophos 40EC), Profenofos (Curacron 500EC), Quinalphos (25%EC), (Master 600 SL) were obtained from local sources and were used for the bioassay.

Lambda-cyhalothrin selection for *M. separate*: A pre-experiment was conducted to ascertain the concentration of selection. Fourth instar larvae were used for bioassay under laboratory conditions with temperature 27±2°C and relative humidity 65±5. Selection at different concentrations was performed so that enough larvae were maintained for further selection. Survivors were reared for the selection process.

***Bioassay*:** Maize leaves were used to assess toxicity of the pesticides lambda-cyhalothrin, chlorpyrifos, triazophos, profenofos, quinalphos, and methamidophos. Six concentrations were used and each was replicated 3 times. Insecticide dilution ranged from 0-10 µg a.i /ml for the susceptible strain of *M. separata*. Dilution ranged from 70-280 µg a.i /ml for the lambda-cyhalothrin selected strain during G_1 to G_8. Fresh leaves of maize aging from 2-3 days were dipped in insecticides for 10 seconds. For one replication, at one concentration, 48 larvae were used. Data were recorded after 48hours.

***Genetic crosses and back cross*:** The lambda-cyhalothrin selected resistant strain was reciprocally crossed to assess genetic mode of resistance. The F_1 progeny was back-crossed to a resistant one, each time 100 males from the resistant strain were crossed to 100 F_1 females. Then 100 males from F_1 were crossed to 100 females of the resistant strain.

***Estimation of degree of dominance*:** *h* value was calculated at specific concentration as

$$h = (W_{10}-W_{20}) / (W_{11}- W_{20})$$

Where W_{10}, W_{11}, W_{20} are fitness of F_1 heterozygous, resistant parents and susceptible parents, respectively. *h* value can be changed from 0-1 (0= completely recessive, 0.5=co-dominance, 1=dominant) (Liu and Tabashnik 1997; Bourguet *et al*., 2000).

***Loci influencing inheritance/ monogenic or polygenic resistance test using chi square*:** A Survival bioassay of the back cross of progeny of the F_1 reciprocal cross was used to determine the number of loci influencing inheritance. The back-cross null hypothesis is that parental RR was crossed reciprocally to SS resulting in progeny F_1, which would be all RS. While back-cross RSXRR would result in equal populations of RS and RR. If the null hypothesis is correct then the expected mortality at concentration X will be calculated as

$$Y_x = (M_{RS}+M_{RR})/2$$

Where RS and RR are F_1 and resistant strains at concentration X.

$$X^2 = (F_1-pn)^2/ pqn \quad \text{(Tabashnik 1991)}$$

P is expected mortality and q=1-p

***Statistical analysis*:** Data were analyzed using probit analysis (Finney, 1971) using POLO Plus software (Software, 2002) to aascertain LC_{50}, Confidence Interval (CI), slopes and Standard Error (SE). The LC_{50} of a reciprocal cross was considered significant when Confidence Interval (CI) did not overlap (Litchfield and Wilcoxon, 1949). Probability calculation was done by chi square method. The 95% Confidence Interval (CI) of RR was calculated according to Robertson and Preisler (1992) and considered significant if did not include 1.

RESULTS

***Selection of resistance to lambda-cyhalothrin*:** Selection of *M. separata* for 14 generations resulted in decreased mortality, 48.83% to 0.30% at 130 to 1200 µg/ml concentration of lambda-cyhalothrin. Selection with lambda-cyhalothrin for 14 generations resulted in an increase in LC_{50} value (ranging from G_1 LC_{50} of 120.67to 1153.80 µg /ml of a.ifor lambda-cyhalothrin selected strain) as compared to susceptible LC_{50} 0.96 µg /ml. RR value was increased from 125.69folds to 1201.87folds as compared to the susceptible (Table 2).

***Field strains and their resistance evaluation*:** As shown in Table 3, two years field (sprayed with lambda-cyhalothrin) collected resistant strains showed that the Resistance Ratio (RR) was high in all districts against lambda-cyhalothrin. For Faisalabad collected samples, LC_{50} was 127.41 and 133.52 µg /ml during 2017 and 2018, respectively. The Resistance Ratio (RR) during 2017 and 2018 was 126.14 and 146.72 folds, respectively. For Multan collected samples, LC_{50} was 130.62

Table 2. Response of *Mythimna separata* to lambda-cyhalothrin at different concentrations.

Selection	LC_{50}	Slope	X^2	df	RR
Susceptible	0.96(0.78-1.09)	0.35±0.21	8.14	16	-
G_1	120.67(97.40-146.32)	2.90±0.30	14.46	16	125.69
G_2	160.58(125.37-209.34)	2.14±0.25	13.14	16	167.27
G_3	192.77(145.23-245.22)	2.54±0.33	9.56	16	200.80
G_4	234.12(188.54-294.03)	3.44±0.67	11.18	16	243.87
G_5	318.21(283.24-384.60)	4.37±0.24	12.17	16	331.46
G_6	454.61(396.53-507.30)	2.06±0.42	12.13	16	473.55
G_7	501.79(456.96-587.41)	3.06±0.38	10.83	16	522.69
G_8	591.16(515.656-78.07)	3.29±0.46	9.54	16	615.79
G_9	670.02(610.34-754.23)	2.29±0.31	10.31	16	697.93
G_{10}	752.57(702.61-794.67)	3.11±0.11	9.57	16	783.92
G_{11}	809.72(776.44-891.33)	2.75±0.45	13.42	16	843.45
G_{12}	913.44(869.39-989.25)	3.03±0.75	11.13	16	951.50
G_{13}	1047.72(995.48-1105.62)	2.65±0.78	12.39	16	1091.37
G_{14}	1153.80(1113.49-1240.42)	2.02±0.41	10.71	16	1201.87

and 132.66 µg /ml during 2017 and 2018, respectively. Resistance Ratio (RR) during 2017 and 2018 was 109.76 and 123.98 folds, respectively. For Vehari collected samples, LC_{50} was 135.22 and 136.4 µg /ml during 2017 and 2018, respectively. Resistance Ratio (RR) during 2017 and 2018 was 148.59 and 128.67 folds, respectively. For Khanewal collected samples, LC_{50} was 129.41 and 132.66 µg /ml during 2017 and 2018, respectively. Resistance Ratio (RR) during 2017 and 2018 was 124.43 and 141.12 folds, respectively (Table 3).

***Cross resistance of lambda-cyhalothrin selected strain of M. separate to organophosphate insecticides*:** The lambda-cyhalothrin field collected population of *M. separata* $G_{2,}$showed low cross resistance to chlorpyrifos (6.74 folds); lower to high cross resistance against triazophos (17 folds) and profenophos (21.64 folds); moderate cross resistance to quinalphos (45.26 folds); and high cross resistance to methamidophos (85.05 folds) as compared to susceptible strains (Table 4).

The lambda-cyhalothrin selected strain of *M. separata* G_{14}showed low to higher cross resistance to chlorpyrifos (19 folds); moderate cross resistance to triazophos (31.74 folds); higher cross resistance to profenophos (59.94 folds); very high cross resistance to quinalphos (69.85 folds); and very higher cross resistance to methamidophos (101.47 folds) as compared to susceptible strain (Table 4).

The lambda-cyhalothrin selected strain of *M. separata* showed very low cross resistance to chlorpyrifos (2.81folds); to triazophos (1.84 folds); to profenofos (2.63 folds), to quinalphos (1.54folds), and to methamidophos (1.19 folds) after 8 generations of selection (G_8) compared to G_2 of the field population (Table 4).

***Maternal effects*:** The LC_{50} of reciprocal crosses with susceptible and resistant parents shown non-significant difference (overlapping of 95% FL), Tables 5 and 6. This

Table 3. Field collected strains from different areas collected in different years from Punjab province Pakistan.

Field Area	Strains	Year	n[a]	LC_{50} (95%FL)(µg/g)	Fit for probit line			RR [b]
					Slope ±SE	χ^2	df (χ^2)	
Faisalabad	Lambda-S	2017	571	1.01 (0.87-1.28)	1.22±0.24	0.87	12	1.00
		2018	510	0.91 (0.74-1.35)	0.87±0.31	1.43	12	1.00
	Lambda -R	2017	525	127.41 (91.24-177.13)	1.71±0.72	1.21	12	126.14
		2018	575	133.52 (92.45-184.32)	0.94±0.33	1.92	12	146.72
Multan	Lambda -S	2017	535	1.19 (0.80-1.50)	1.51±0.72	0.98	12	1.00
		2018	578	1.07 (0.83-1.57)	1.21±0.11	0.44	12	1.00
	Lambda -R	2017	560	130.62 (98.76-188.03)	1.13±0.32	0.49	12	109.76
		2018	510	132.66 (101.23-183.41)	1.74±0.64	0.87	12	123.98
Vehari	Lambda -S	2017	525	0.91 (0.57- 1.43)	1.04±0.56	0.66	12	1.00
		2018	505	1.06 (0.69- 1.62)	0.76±0.20	0.50	12	1.00
	Lambda -R	2017	540	135.22 (82.93- 187.6)	1.43±0.24	1.20	12	148.59
		2018	520	136.40 (94.21-190.92)	1.31±0.84	1.04	12	128.67
Khanewal	Lambda -S	2017	530	1.04 (0.74-1.54)	1.23±0.44	0.76	12	1.00
		2018	510	0.94 (0.68-1.62)	1.17±0.28	1.36	12	1.00
	Lambda -R	2017	508	129.41(83.35-177.4)	0.87±0.60	0.78	12	124.43
		2018	545	132.66(92.24-179.51)	1.21±0.81	1.20	12	141.12

Table 4. Cross resistance of insecticides, in field populations, and lambda-cyhalothrin selected populations of *Mythimna separata.*

Strain	Insecticide	LC_{50}	RR[a]	RR[b]	Slope	X^2	df
Susceptible	Lambda-cyhalothrin	1.37(1.03-1.67)	-		5.06±0.35	70.86	16
Field-selected (G_2)	Chlorpyrifos	9.24(8.45-10.06)	6.74	1.00	6.23±0.44	9.22	16
	Triazophos	23.58(21.07-26.18)	17.21	1.00	7.49±0.48	28.73	16
	Profenofos	29.65(25.32-33.91)	21.64	1.00	7.08±0.50	34.84	16
	Quinalphos	62.01(58.50-65.21)	45.26	1.00	11.45±1.04	14.59	16
	Methamidophos	116.53(103.87-128.68)	85.05	1.00	5.81±0.40	28.16	16
Lambda-cyhalothrin	Chlorpyrifos	26.04(18.75-33.47)	19.00	2.81	6.23±0.42	133.70	16
selected (G_{14})	Triazophos	43.49(38.48-48.06)	31.74	1.84	7.89±0.60	34.81	16
	Profenofos	78.01(74.22-81.84)	59.94	2.63	9.88±0.67	14.37	16
	Quinalphos	95.70(85.89-105.55)	69.85	1.54	3.75±0.21	32.97	16
	Methamidophos	139.02(128.75-149.02)	101.47	1.19	7.74±0.51	22.26	16

RR[a]= Field/lab. selected resistant/ susceptible strain; RR[b]=Lab. selected resistant strain/ field collected resistant population

Table 5. Reciprocal crosses between resistant and susceptible strains of *Mythimna separata.*

Strain	LC_{50}	Slope	X^2	df
Susceptible	1.88(1.54-2.13)	8.07±0.79	47.38	16
Lambda-cyhalothrin-Sel♂ X S♀	126.64(94.90- 188.44)	5.71±0.38	18.32	16
S♂ X lambda-cyhalothrin-Sel♀	125.24(92.16- 194.49)	5.12±0.31	28.49	16

Resistance is considered significantly different if the LC_{50} does not overlap on 95% FL and not significantly different if the LC_{50} overlap on 95%FL.

Table 6. F_1 progeny back-cross with susceptible parents.

Strain	LC_{50}	Slope	X^2	df	RR
Susceptible	1.26(0.60-1.81)	5.34±0.62	12.04	5	-
F_1♀(S♀xlambda-sel♂)x SS♂	15.02(6.15-37.62)	4.29±0.48	16.16	5	
F_1♂(S♂xlambda-sel♀)x SS♀	16.35(6.26-37.79)	4.52±0.50	13.86	5	
SS♀xF_1♂(S♂xlambda-sel♀)	16.73(8.80-37.46)	3.64±0.41	18.96	5	
SS♂xF_1♀(S♀ xlambda-sel♂)	17.56(6.72-41.94)	5.26±0.59	18.29	5	

indicates that lambda-cyhalothrin resistancewas autosomal and has no maternal effects.

Loci influencing inheritance/ monogenic or polygenic test using chi-square: Calculated and expected mortality, to determine polygenic/ monogenic mode of inheritance of resistance, shows that non-significant differences are lower at all concentrations, thus there are multiple factors controlling resistance to lambda-cyhalothrin in *M. separata* (Table 7).

Table 7. Monogenic model for actual and expected mortality.

Strain	Actual mortality (%)	Expected Mortality (%)	χ^2
F_1♀(S♀xlambda-cyhalothrin-sel♂)x SS♂			
5	12.50	8.20	0.97
15	25.00	16.41	1.26
50	72.91	58.12	9.36
100	100.00	74.00	9.26
F_1♂(S♂xlambda-cyhalothrin -sel♀)x SS♀			
5	10.41	6.66	0.49
15	22.91	14.87	1.08
50	75.00	59.66	9.54
100	100.00	74.00	9.26
SS♀xF_1♂(S♂xlambda-cyhalothrin -sel♀)			
5	14.58	9.75	0.86
15	27.08	17.95	4.91
50	77.08	61.20	9.73
100	100.00	74.00	9.26
SS♂xF_1♀(S♀ x lambda-cyhalothrin -sel♂)			
5	10.41	6.66	1.52
15	20.83	14.37	1.84
50	70.83	60.75	13.70
100	100.00	74.00	9.26

Dominance: The results of dominance showed that the level of dominance increased when lambda-cyhalothrin concentration decreased. The. *h* value shows that resistance was expressed as an incomplete dominant trait in reciprocal cross progenies (Table 8). Resistance was incomplete dominant at lower concentrations and incomplete recessive at higher concentrations (Table 8).

Table 8. Effective dominance of bifenthrin selected resistance in *M. separata*

Conc.	Strain	Survival%	Fitness	*h*
	Susceptible	83.33	0.83	
0.5	lambda-cyhalothrin-sel	100.00	1.00	1.00
	F_1	100.00	1.00	
	Susceptible	0.00	0.00	
5.0	lambda-cyhalothrin-sel	100.00	1.00	0.90
	F_1	81.25	0.90	
	Susceptible	0.00	0.00	
50	lambda-cyhalothrin-sel	92.80	1.00	0.63
	F_1	14.58	0.63	
	Susceptible	0.00	0.00	
100	lambda-cyhalothrin-sel	64.72	1.00	0.00
	F_1	0.00	0.00	

DISCUSSION

Pyrethroid insecticides, including lambda-cyhalothrin, are broad-spectrum pesticides, which work on sodium channels sensitive to voltage (Smith *et al.*, 2018). In our experiments, insects collected from the field showed 126 folds of resistance. Lower to moderate levels of resistance to lambda-cyhalothrin might be due to resistance developed as a result of sprays in the field for *M. separata* and other insects. Further laboratory studies showed higher levels of resistance. It is evident that resistance development is an evolutionary process resulting from continuous selection pressure in resistant insect populations. Based on our results we presumed that resistance developed in *M. separata* might be due to genetic characteristics at the species level favoring expression of resistant genes (Alvarez *et al.*, 2013).

Our results are in agreement with those of Ijaz and Shad. (2018). In our experiments, resistance development was found after 8 generations of selection in *M. separata*. Our cross resistance results showed that the lambda-cyhalothrin selected strain of *M. separata* exhibited the least cross resistance against chlorpyrifos. These results were in agreement with Ismail those of *et al.*(2017), in which chlorpyrifos selected strains of insect were found to show less cross-resistance to lambda-cyhalothrin and profenofos. We differ with those results as our results for profenofos, show moderate cross resistance against lamda-cyhalothrin selected *M. separata*. This might be due to the different insect species studied in our experiments.

A cause of polygenic resistance is continuous selection pressure of insecticides (Roush, 1998). Back-cross experiments results allowed us to reject the null hypothesis of monogenic mode of resistance in *M. separata*. According to Barnes *et al.* (1995) resistance selection of insecticides is determined by dominant type of gene by allele reshuffling and is expressed as dominant. In our experiments, resistance was incomplete dominant at lower concentrations while completely recessive at higher concentrations.

In conclusion, this study revealed increased rates of resistance development to lambda-cyhalothrin in the selected strain of *M. separata.* Resistance development, under selection pressure in laboratory, showed that unlike resistance development in the field, selection pressure to lambda-cyhalothrin will persist for long period of time. Insecticides like chlorpyrifos, which show lower cross resistance, can be used in Integrated Resistance Management (IRM) programs to suppress *M. separata.* In addition, combining biochemical and genetic mechanisms of resistance to lambda-cyhalothrin and other insecticides needs further research.

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