TOBACCO BUDWORM RESISTANCE TO PYRETHROIDS: RESISTANCE SPECTRA, SYNERGISTS, AND SUBSTITUTE INSECTICIDES

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Abstract

First and third instar larvae and adult males of susceptible (S) and pyrethroid (PY) resistant (R) strains of tobacco budworm (TBW), *Heliothis virescens* (F.), were bioassayed with different insecticides using a glass liquid scintillation vial technique. Resistance to the PYs cypermethrin and fenvalerate was observed in first instar larvae of all R strains. Low or no tolerance by first instar larvae was observed to the organophosphates profenofos, acephate, and methyl parathion or the cyclodiene endosulfan. Only one R strain, Uvalde, showed resistance to the oxime carbamate thiodicarb. The combination cypermethrin plus thiodicarb seemed to be synergistic only against the Uvalde strain. Overall, the resistance spectra to different insecticides were approximately the same for different R TBW strains. Chordimeform synergized all insecticides tested against first instar larvae. The synergism was variable for different insecticides between strains. Differences in tolerance to cypermethrin observed between R adult males and first instar larvae showed that resistance does not manifest equally in all developmental stages of TBW. Tests with cypermethrin and methyl parathion showed higher resistance in third than in first instar larvae, indicating the presence of both target site and metabolic resistances. On the other hand, no stage showed tolerance to the ± isomer organophosphorothiolate acephate. The use of 3-way combination of cypermethrin, chlordimeform, and piperonyl butoxide gave nearly 2,000-fold synergism at the LC-90 level and improved the control of PY-R third instar TBW larvae to such an extent that toxicity was only about 6-fold less than that of the cypermethrin only against the susceptible strain. Based on the information presented, a general approach for managing PY resistance in TBW is proposed.

Introduction

Failures of tobacco budworm (TBW), *Heliothis virescens* (F.), control in the field with pyrethroid (PY) insecticides have been reported for several years, most notably in California and Arizona (Twine and Reynolds 1980, Martinez-Carillo and Reynolds 1983, Crowder et al. 1984).

The first serious control problems with PYs in Texas were reported in the Uvalde, Glasscock Co., and Fort Stockton areas in 1985 (Plapp and Campanhola 1986). An approximately 16-fold resistance to permethrin was observed in first instar larvae. Chlordimeform (CDF) added to permethrin was synergistic and largely overcame resistance.

Problems of TBW control with PYs occurred in several cotton production areas during the 1986 season. An adult monitoring program performed during that year in association with failures of control in the field confirmed the existence of TBW resistance in Texas (Allen et al. 1987, Plapp et al. 1987), Arkansas (J. R. Phillips, personal communication), Mississippi (Roush and Luttrell 1987), and Louisiana (Leonard et al. 1987). Control problems continued to be observed in the 1987 season in some areas. Probably the adoption of management strategies by the growers was responsible for ameliorating the situation as compared to 1986 (Plapp et al. 1988).

In 1986, susceptible (S) and resistant (R) first instar TBW larvae were bioassayed with different insecticides (Campanhola and Plapp 1987). The same R population ICI US-83 was used in the present study. Since the resistance extended to all the PYs, it can be assumed that the PY resistance observed is of the kdr type, a target-site-change resistance, due to a recessive gene (Plapp and Wang 1983).

The use of synergized insecticides may prove useful in counteracting the development of resistance (Wilkinson 1976, Oppennorth 1971). CDF, a formamidine, has been used previously and showed to be a good synergist when combined with organophosphate (OP) and PY insecticides against TBWs (Plapp 1976, Plapp 1979, Campanhola and Plapp 1987). Piperonyl butoxide, a methylenedioxyphenyl, is known to inhibit the mixed-function oxidases and is an effective synergist for several insecticides towards both S and R strains of insects (Wilkinson 1983).

The S-alkyl-phosphorothiolate insecticides and endosulfan may constitute alternate chemicals for the control of PY-R TBWs. In a previous study, slight or no tolerance was observed to sulprofos, profenofos, and acephate by PY-R first instar TBW larvae (Campanhola and Plapp 1987). Endosulfan, a biodegradable and very cheap cyclodiene, was recommended for controlling *Heliothis armigera* in Australia, early and mid season, as part of the strategy for managing resistance to PYs (Sawicki 1985). However, it is not clear if there is cross resistance to endosulfan in PY-R strains of *H. armigera*, since controversial results were obtained by N. Forester and P. Twine (Sawicki 1985).

The objectives of the present study were to determine the resistance spectra for different TBW strains, to determine alternate insecticides or insecticide-synergist combinations for the control of PY-R TBWs, and to establish the relationships between the resistance levels in different developmental stages of TBW as well as to understand the mechanisms of resistance involved. Based on the results obtained, we present recommendations for the management of resistance in TBW.

Materials and Methods

Insects

The S and R TBW strains for the bioassays were obtained from lab colonies maintained on artificial diet (Vanderzant et al. 1962). The S strain (Stoneville) was provided by the Southern Field Crop Insect Management Laboratory, USDA, ARS, Stoneville, MS, where it has been reared for several years without exposure to insecticides. Three R strains were studied and designated ICI, Uvalde, and Hearne. The ICI strain (US-83) was prepared by ICI Americas, Goldsboro, NC, from a mixture of 10 different populations collected from cotton fields in different states. The resistance was developed by in-house permethrin pressure for several generations. The other two R TBW strains were brought to the lab from cotton fields where control failures with PYs were observed. The Uvalde strain was collected near Uvalde, TX by D. F. Clower, consultant for ICI Americas, in July, 1986. The Hearne strain was provided by V. V. Turner, a private consultant, and was collected near Hearne, TX, in August, 1986.

Chemicals

All the chemicals were supplied by commercial sources as technical grade materials. Against first instar larvae, the insecticides used included the PYs cypermethrin and fenvalerate, the OPs methyl parathion, profenofos, and acephate, the carbamate thiodicarb, the combination cypermethrin plus thiodicarb, and the cyclodiene endosulfan. Against third instar larvae, cypermethrin, methyl parathion, and acephate were tested. The compounds used as synergists were the formamidine CDF and the mixed-function oxidase inhibitor piperonyl butoxide (PB).

Bioassays of Larvae

First instar TBW larvae were exposed to films of chemicals on the inner surfaces of 20-ml glass liquid scintillation vials (Plapp 1971). A piece of artificial diet and five larvae were placed in each vial. Thereafter the vials were plugged with cotton. All the insecticides were tested against S and R TBW populations. Insecticide(s) plus CDF were tested at a 1:10 (wt/wt) ratio and the cypermethrin plus thiodicarb combination, at a 1:1 (wt/wt) ratio. Four or five different concentrations were used for each insecticide or insecticide combination in addition to untreated controls (acetone only). Readings for mortality were conducted 24 hr. after the exposure started.

Third instar larvae from the S and ICI populations were also bioassayed. Basically the same procedure used for first instar larvae was adopted. However, the readings for mortality were performed after 72 hours and only one larva was bioassayed per vial. In addition, the effects of PB in combination with cypermethrin or cypermethrin plus CDF were evaluated.

Bioassays of Adults

The same vial technique described for first instar larval bioassays was used to measure the response of R and S adult male TBWs to cypermethrin, methyl parathion, and acephate. At least four different concentrations were used for each insecticide. Two male moths were tested per vial containing a small piece of cotton wick soaked with 10% sucrose solution in water.

Data Analyses

LC$_{50}$s, in μg toxicant per vial, as well as slopes of the response curves were calculated by probit analysis (SAS Institute 1982). Data from all bioassays were corrected for control mortality using Abbott's formula (Abbott 1925). Overall control mortality ranged from 0 to 10%. The resistance levels were determined dividing the LC$_{50}$ of each toxicant for the R strain by the LC$_{50}$ for the S strain. The synergism levels due to CDF and/or PB were calculated by dividing the LC$_{50}$ for the insecticide only by the LC$_{50}$ for the insecticide plus synergist(s). The synergistic effect for the combination cypermethrin plus thiodicarb was evaluated by cotoxicity coefficients (Sun and Johnson 1960).

Results and Discussion

The results of toxicity tests for the insecticides, alone or combined with CDF, against first instar larvae of different TBW strains are presented in Table 1. The resistance ratios and CDF synergism are presented in Table 2.

Resistance was present to both PYs tested, cypermethrin and fenvalerate. Furthermore, in tests with some of the R TBW strains it was observed that R was widespread to all the pyrethroids (data not shown). This is a characteristic of target-site resistance, that is, a change in the number of sites available for insecticide binding (Chang and Plapp 1983). The resistance levels varied from 10.9- to 52.2-fold for cypermethrin and from 9- to 47.8-fold for fenvalerate. However, there was no consistent resistance pattern to these compounds. Resistance was greater in the ICI strain for fenvalerate than for cypermethrin. However, for the Hearne strain, the resistance level was similar to both insecticides. Leonard et al. (1987) also found differences in susceptibility to these insecticides in third instar TBW larvae from different R field populations.

CDF synergism was higher with cypermethrin than with fenvalerate. Also CDF synergized cypermethrin more against the R strains than against the S strain. With fenvalerate CDF synergism was similar against all the strains studied.

Low or no tolerance in first instar larvae was observed to the OPs profenofos, acephate, and methyl parathion. Previous study showed the presence of low resistance to sulprofos in the ICI strain (Campanhola and Plapp 1987). The S-alkyl-phosphorothiolates, sulprofos, profenofos, and acephate, are relatively safe for natural enemies (Plapp and Vinson 1977, Plapp and Bull 1978). They have plus or minus isomers due to the presence of four different substituents (Plapp 1986); therefore, metabolic resistance present to other OPs does not usually extend to compounds of this type.

CDF synergized all OPs against all strains. However, the only appreciable level of synergism was observed for acephate plus CDF against the S strain (42.4-fold). Also, synergism tended to be higher against the S than against the R strains when CDF was combined with these insecticides. Therefore, the resistance level increased when CDF was combined with OPs. Nevertheless, CDF increased the toxicity of these chemicals to R larvae, making them equally or more toxic to R larvae than the insecticide only to S larvae. Another advantage of adding CDF to OPs for R TBW control is an overall decrease in the slope of the response curves, which may minimize resistance development (Plapp et al. 1979).

For years methyl parathion was used for Heliothis control in cotton. In IPM programs, methyl parathion is too disruptive of natural enemies and may cause pest outbreaks when used early in the season. Another restriction for the widespread use of methyl parathion as a PY-alternate insecticide is that TBW resistance to this compound was previously observed throughout the cotton belt (Wolfenbarger and McGarr 1970, Graves et al. 1973, Pieters and Boyette 1977, Crowder et al. 1979, Twine and Reynolds 1980) and selection pressure might easily select for resistance again. Therefore, among the OPs, the S-alkyl phosphorothiolates are possible alternate insecticides to the PYs where resistance to the latter is present.

Uvalde first instar larvae showed substantial resistance to the oxime carbamate thiodicarb, but Hearne and ICI larvae were more susceptible to the insecticide than the S strain. In previous tests, with the ICI strain, a 120-fold resistance was observed in first instar larvae (Campanhola and Plapp 1987). The results listed here are from a new sample of the ICI strain. It is not clear why the resistance level changed and if there is a cross resistance relationship for PYs and oxime carbamates.

CDF synergism with thiodicarb was very high for the S and Uvalde strains, with synergism levels of 55.7- and 300.2-fold, respectively. For the other two R strains, ICI and Hearne, synergism was 4.4- and 2.6-fold, respectively. Here also, even though CDF did not block resistance completely, it increased thiodicarb toxicity to R larvae to a level greater than that observed for the insecticide only against the S larvae. CDF did not change consistently the slope of response curves to thiodicarb. For the S and Uvalde strains the curves became flatter with the addition of CDF, which is advantageous in terms of resistance management, but the opposite occurred with ICI and Hearne strains.

The cotoxicity indices for the combination cypermethrin plus thiodicarb were 0.2, 10.2, and 1.4 for the ICI, Uvalde, and Hearne strains, respectively. Therefore, this combination seemed to be synergistic only against the Uvalde strain. CDF synergized this combination more against the R strain than against the S strain, with as much as 72.8-fold synergism observed for the ICI strain. With the R strains, no significant changes were observed in the slope of the response curves with the addition of CDF. The combinations cypermethrin plus thiodicarb and cypermethrin plus thiodicarb plus CDF were tested in a cotton field in Uvalde, TX and were included among the best treatments for TBW and bollworm control (C. T. Allen, unpublished data).

First instar TBW larvae showed, practically, no tolerance to endosulfan. Against the ICI strain, endosulfan was even more toxic than against the S strain. Thus, there seems to be no cross resistance between the PYs and endosulfan in TBW. CDF synergism with endosulfan was higher against S than against R strains. Thus, there seems to be not much advantage in combining CDF with endosulfan for control of PY-R TBWs. However, when CDF was combined with this toxicant, the LC_{50} for the R strains became nearly equal or lower than the LC_{50} for the insecticide only against the S strain. Therefore, endosulfan alone or combined with CDF can be another alternative for controlling PY-R TBWs.

In summary, the resistance spectra for all insecticides were approximately the same for the different R strains. CDF synergized all insecticides tested, but the synergism level was variable for different insecticides against different R strains. The results showed that the S-alkyl phosphorothiolates profenofos and acephate, alone or combined with CDF, the carbamate thiodicarb plus CDF, endosulfan ± CDF, and the combination cypermethrin plus thiodicarb plus CDF are possible alternate toxicants for PY-R TBW control.

Data on cypermethrin toxicity against TBW adult males of different strains are shown in Table 3. These results were compared to those obtained for first instar larvae (Table 1). For all strains, adults were 5- to 30-fold more tolerant to cypermethrin than first instar larvae. The R level in male moths ranged from 6-fold for the ICI strain to 17-fold for the Hearne strain. However, no consistent correlation was observed between the resistance levels in first instar larvae and adults. The Hearne strain showed the least resistant first instar larvae, but the most resistant adult males. This may imply physiological and biochemical differences and, consequently, that resistance mechanisms do not manifest equally in all developmental stages of TBW.

Table 4 shows the toxicity and resistance ratios for cypermethrin, methyl parathion, and acephate against three different stages of the ICI and S strains. Much higher LC_{50}s were observed for third instar larvae exposed to cypermethrin and methyl parathion in the ICI strain than in the S strain. Also tolerance was observed in the ICI adults treated with cypermethrin and methyl parathion. On the other hand, with acephate the toxicity varied very little for different stages of both TBW strains. Preliminary results have shown the same pattern for other S-alkyl phosphorothiolates such as profenofos and sulprofos.

First instar larvae showed practically no tolerance to methyl parathion (1.8-fold), but showed tolerance to cypermethrin (20.3-fold). This is evidence for the presence of target-site resistance in that stage. Conversely, the 19-fold resistance to methyl parathion and 998-fold to cypermethrin demonstrated by third instar larvae is evidence for metabolic resistance. Metabolic resistance seems also to be present in adults due to some tolerance of this stage to methyl parathion. On the other hand, all stages showed no tolerance to acephate. It seems, therefore, that there are two genes responsible for resistance in the ICI strain, one for target-site resistance and the other for metabolic resistance. These results agree with those obtained previously where it was suggested the presence of two genes in permethrin-R TBW responsible for the above resistance mechanisms (Payne et al. 1987).

The effects of combining cypermethrin with CDF and/or PB against ICI third instar larvae are presented in Table 5. Synergism was observed with all the combinations tested. At the LC_{50} level, synergism was 77.1-fold for cypermethrin plus CDF, 159-fold for cypermethrin plus PB, and 257.6-fold for cypermethrin plus both CDF and PB. At the LC_{90} level, the treatments cypermethrin plus PB and cypermethrin plus CDF plus PB showed very high synergism with levels of 1740.5- and 1946.7-fold, respectively. Thus, the combinations cypermethrin plus PB and cypermethrin plus CDF plus PB can improve the control of PY-R third instar TBW larvae, since their toxicities were only about 6-fold less than that of the cypermethrin only against the susceptible strain. The reversal of insecticide resistance by PB constitutes a useful indicator of the extent of mixed-function oxidase involvement in insecticide resistance (Wilkinson 1983). Thus, the synergism by PB reinforce the hypothesis for the presence of metabolic resistance in PY-R TBWs. However, since TBW seems to show more than one mechanism of resistance, the use of combinations such as cypermethrin plus PB may block one metabolic pathway and select for resistance by other mechanism(s) (Wilkinson 1983). Therefore, only the combination cypermethrin plus CDF plus PB seems to be a good alternative for controlling PY-R third instar TBW larvae.

Very little is known about the effect of synergists on the onset of resistance. In favor of the use of synergists there are data showing that the use of CDF in combination with pyrethroids may prevent the development of resistance. After 10 generations of selection with a 1:1 permethrin:CDF ratio, at the LD_{80} level, the susceptibility to permethrin did not change in the population of TBW tested (Crowder et al. 1984). In contrast, selection with permethrin only during 11 generations raised the LD_{50} 37-fold compared with the LD_{50} of the F_1.

Recommendations

Based on all the data presented, a general scheme for resistance management can be proposed. The main point is to delay the onset of resistance by one generation, early in the season, that is, to displace the build up of resistance from June to July so that the R gene frequency would not reach high levels until late in the season, when the yield is already assured. The idea is to avoid the use of PYs early in the season, minimizing the selection pressure and increasing the efficacy of these insecticides during the critical mid season period. Therefore, the S-alkyl phosphorothiolates profenofos, sulprofos, and acephate, the cyclodiene endosulfan and the oxime carbamate thiodicarb, alone or combined with CDF, are possible alternate insecticides for the control of PY-R first instar TBW larvae, particularly early in the season. If later instar larvae are to be controlled, the S-alkyl phosphorothiolates are probably the best choice.

Late season control of TBW tends to be more difficult to achieve. If control is required at this time, it is desirable to use other types of insecticide than PYs. The same insecticides mentioned above for early season control could be utilized later in the season for controlling PY-R first instar TBW larvae. Again, the combination with synergists such as CDF would enhance the efficacy of those insecticides against R TBWs. Also, the combination of PYs plus CDF plus PB seemed to be very effective in suppressing later instar larvae of PY-R TBWs. To date there are no field comparisons between this combination and alternate insecticides. Therefore, it is not clear which is the best approach for controlling late season insects under field conditions.

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Table 1. Toxicity of insecticides ± chlordimeform (CDF) against susceptible (S) and resistant (R) first instar tobacco budworm larvae.

Insecticide	Stoneville (S)			ICI (R)			Uvalde (R)			Hearne (R)		
	n[1]	slope	LC$_{50}$ (95% CL)[2]	n	slope	LC$_{50}$ (95% CL)	n	slope	LC$_{50}$ (95% CL)	n	slope	LC$_{50}$ (95% CL)
Cypermethrin	189	1.96	0.15 (0.11-0.19)	254	1.16	3.04 (2.15-4.31)	195	0.84	7.83 (4.06-12.99)	365	1.24	1.63 (1.23-2.13)
Cyp. + CDF	260	0.75	0.008 (0.004-0.01)	247	0.92	0.19 (0.11-0.29)	164	1.07	0.20 (0.092-0.31)	80	1.78	0.055 (0.0032-0.11)
Fenvalerate	245	1.14	0.24 (0.17-0.34)	230	1.11	11.5 (7.74-16.51)	-[3]	-	-	210	1.36	2.17 (1.49-2.97)
Fenv. + CDF	180	1.28	0.04 (0.028-0.06)	189	1.47	1.31 (0.9-1.78)	-	-	-	165	2.41	0.59 (0.43-0.74)
Profenofos	148	1.93	0.084 (0.054-0.12)	133	2.31	0.21 (0.16-0.27)	264	1.51	0.15 (0.039-0.29)	105	1.68	0.18 (0.12-0.27)
Prof. + CDF	194	1.47	0.02 (0.015-0.028)	150	1.12	0.044 (0.03-0.07)	233	1.67	0.05 (0.036-0.06)	170	1.59	0.079 (0.058-0.11)
Acephate	185	1.61	4.66 (3.38-6.29)	245	1.48	2.36 (0.13-6.55)	256	0.68	9.16 (5.38-19.32)	150	1.09	6.26 (3.74-10.04)
Aceph. + CDF	233	0.67	0.11 (0.033-0.22)	125	1.77	0.72 (0.50-0.99)	233	1.60	1.74 (1.26-2.69)	125	1.38	0.57 (0.32-0.86)
M. Parathion	200	1.01	0.16 (0.096-0.24)	145	1.45	0.29 (0.13-0.43)	100	1.78	0.21 (0.13-0.29)	130	3.06	0.065 (0.053-0.08)
M. Par. + CDF	321	0.77	0.016 (0.09-0.025)	100	1.20	0.20 (0.071-0.32)	100	1.34	0.064 (0.02-0.1)	100	2.00	0.032 (0.017-0.045)
Thiodicarb	198	1.03	1.95 (1.22-2.99)	315	0.53	0.74 (0.27-1.37)	126	0.91	33.02 (18.6-90.6)	125	0.66	1.01 (0.0001-3.28)
Thiod. + CDF	285	0.97	0.035 (0.023-0.05)	100	2.17	0.17 (0.11-0.23)	269	0.82	0.11 (0.01-0.27)	104	2.22	0.39 (0.24-0.52)
Cyp. + Thiod.	90	3.54	0.085 (0.068-0.11)	95	0.99	2.91 (1.15-5.9)	175	1.07	0.62 (0.12-1.22)	150	1.11	0.45 (0.25-0.71)
Cy. + Th. + CDF	165	1.56	0.028 (0.02-0.038)	75	1.02	0.04 (0.001-0.09)	169	0.97	0.058 (0.02-0.1)	125	1.28	0.093 (0.057-0.15)
Endosulfan	290	2.01	1.22 (1.01-1.48)	185	1.28	0.53 (0.32-0.76)	150	1.61	3.47 (2.52-5.05)	120[4]	2.72	1.11 (0.86-1.41)
Endos. + CDF	101	1.91	0.069 (0.038-0.099)	200	1.66	0.12 (0.082-0.16)	-	-	-	195[4]	1.37	0.65 (0.46-0.91)

[1] Number of larvae tested excluding controls.

[2] Concentrations are expressed in micrograms of insecticide per vial.

[3] Data not available due to loss of strain.

[4] Hearne strain after 10 generations in the lab (R ratio to cypermethrin = 3).

Table 2. Resistance ratios and chlordimeform (CDF) synergism for different insecticides against susceptible (S) and resistant (R) first instar tobacco budworm larvae

Insecticide	Stoneville (S)	ICI (R)		Uvalde (R)		Hearne (R)	
	Synergism[1]	R ratio[2]	Synergism	R ratio	Synergism	R ratio	Synergism
Cypermethrin		20.3		52.2		10.9	
Cyp. + CDF	19.2	24.4	16.0	25.6	39.2	7.1	29.6
Fenvalerate		47.9		-		9.0	
Fenv. + CDF	6.0	32.8	8.8	-	-	14.8	3.7
Profenofos		2.5		1.8		2.1	
Prof. + CDF	4.2	2.2	4.8	2.5	3.0	3.9	2.3
Acephate		0.5		2.0		1.3	
Aceph. + CDF	42.4	6.5	3.3	15.8	5.3	5.2	11.0
M. Parathion		1.8		1.3		0.4	
M. P. + CDF	10.0	12.5	1.5	4.0	3.3	2.0	2.0
Thiodicarb		0.4		16.9		0.5	
Thiod. + CDF	55.7	4.9	4.4	3.1	300.2	11.1	2.6
Cyp. + Thiod.		34.2		7.3		5.3	
Cy. + Th. + CDF	3.3	1.4	72.8	2.1	10.7	3.3	4.8
Endosulfan		0.4		2.8		0.9	
Endos. + CDF	17.7	1.7	4.4	-	-	9.4	1.7

[1] Calculated by dividing the LC_{50} for the insecticide by the LC_{50} for the insecticide + CDF.

[2] Calculated by dividing the LC_{50} for the resistant strain by the LC_{50} for the susceptible strain.

Table 3. Responses of different strains of adult-male tobacco budworm to cypermethrin.

Strain	n[1]	Slope	LC_{50} (95% CL)[2]	R ratio[3]
Stoneville	119	1.63	2.95 (2.07-4.43)	-
ICI	251	1.93	18.46 (15.11-24.08)	6.3
Uvalde	144	2.02	40.63 (28.31-80.61)	13.8
Hearne	35	3.32	50.38 (35.24-90.66)	17.1

[1] Number of moths tested excluding controls.

[2] Concentrations are expressed in micrograms of insecticide per vial.

[3] Calculated by dividing the LC_{50} for the resistant strain by the LC_{50} for the susceptible strain (Stoneville).

Table 5. Effects of chlordimeform (CDF) and piperonyl butoxide (PB) on cypermethrin against ICI third instar TBW larvae.

Insecticide	n	slope	LC_{50} (95% CL)	Synergism	LC_{90} (95% CL)	Synergism
Cypermethrin	125	0.78	1,288.0 (594-3,325)	-	55,871.0 ($11,362-4.2 \times 10^7$)	-
Cyp. + CDF	100	1.61	16.7 (8.2-24.8)	77.1	104.9 (61.5-385.3)	532.6
Cyp. + PB	120	2.14	8.1 (5.9-10.8)	159.0	32.1 (21.5-64.2)	1,740.5
Cyp.+CDF+PB	108	1.68	5.0 (3.5-7.6)	257.6	28.7 (15.4-109.3)	1,946.7
Cyp. (S strain)	145	2.02	1.3 (0.9-1.7)	-	5.6 (3.9-10.1)	-

Table 4. Toxicity and resistance ratio of insecticides against different stages of the S and ICI (R) TBW strains.

Stage	Cypermethrin			Methyl Parathion			Acephate		
	n	LC_{50} (95% CL)	R ratio	n	LC_{50} (95% CL)	R ratio	n	LC_{50} (95% CL)	R ratio
First instar larvae									
S	189	0.15 (0.11-0.19)	1.0	200	0.16 (0.096-0.24)	1.0	185	4.66 (3.38-6.29)	1.0
ICI	254	3.04 (2.15-4.31)	20.3	145	0.29 (0.13-0.43)	1.8	245	2.36 (0.13-6.55)	0.5
Third instar larvae									
S	145	1.29 (0.92-1.71)	1.0	149	12.57 (8.57-17.15)	1.0	135	22.24 (16.30-30.09)	1.0
ICI	125	1,288.0 (594-3,325)	998.4	174	237.80 (171.63-321.63)	18.9	40[1]	36.15 (17.04-349.74)	1.6
Adult males									
S	119	2.95 (2.07-4.43)	1.0	127	41.10 (31.19-53.02)	1.0	79	13.96 (10.51-18.60)	1.0
ICI	251	18.46 (15.11-24.08)	6.3	94	215.30 (148.4-347.0)	5.2	90	17.58 (12.51-25.54)	1.3

[1] Preliminary results.