RHÔNE-POULENC AG CO., MONITORING PROGRAM: PERFORMANCE OF LARVIN® BRAND THIODICARB AND FOUR OTHER INSECTICIDES AGAINST TOBACCO BUDWORM COLLECTED FROM DIFFERENT SITES IN THE USA COTTON BELT

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Abstract

The resistance ratios we calculated in 1995 showed the larvae collected from sites in the southeast and southwestern part of the cotton belt to have <4 fold tolerance to Larvin. Those obtained from sites in the mid-south demonstrated <12 fold resistance. On the other hand, resistance to the synthetic pyrethroids Sherpa and Decis in these areas ranged from 5 to 44 fold. These results indicated that tobacco budworm larvae possessed significantly higher levels of resistance to Sherpa and Decis than to Larvin. These data suggest that high resistance to synthetic pyrethroids will not cause high cross-resistance to carbamate insecticides.

Larvin proved to be significantly more effective than Methomyl against *Heliothis virescens* larvae obtained from different sites in the cotton belt. We monitored the resistance ratios of Larvin at different times during the cotton growing season. The values obtained early and late in the seasons were lower than those obtained in the middle of the season.

The results we accumulated in the last six years showed gradual improvement in the toxicity of Larvin to tobacco budworm larvae collected from Texas. A comparison between 1994 and 1995 results detected a reduction in Larvin performance against larvae collected from Arkansas and Louisiana. This reduced larvicidal activity will be offset by the good toxicity against the eggs and adults collected from these states.

Introduction

The wide spread of insect resistance to insecticides (Georghiou and Mellon 1983) can cause significant reduction in the activity of pesticides. This prompted several agrochemical companies to initiate research programs to find solutions for this important problem (Riley S. L. 1988 and Lasota et al. 1996). In 1989, we started a program to monitor the insecticidal activity of Larvin against populations of *H. virescens* collected from sites in Louisiana, Texas and North Carolina (Ayad and Phillabaum, 1990). One objective of this program was to establish baseline data to be used for future comparisons. Another objective was to determine if resistance to different classes of insecticides in field populations of *H. virescens* will have an adverse effect on the insecticidal activity of Larvin. Since then, we have expanded the program to include other sites and we have added Sherpa, Bolstar, Methomyl and Decis to the list of insecticides being monitored. In this report, we discuss the results we generated in 1995.

Materials and Methods

Bioassays

The foliar spray method (Payne et al. 1966 and Weiden et al. 1967) was slightly modified and used to assess the toxicity of the insecticides used in this study. The test concentrations were obtained by diluting the formulated insecticides with the appropriate amount of water. We used the turntable sprayer to spray cotton plants to run-off. A volume of 100 ml of each concentration of the test compound was used. After application, we allowed the plants to air dry. Second instar *H. virescens* larvae were individually placed into plastic petri dishes containing a oistened filter paper and one of the treated cotton leaves. Ten larvae were used for each concentration and at least five concentrations were used for each insecticide. The dishes were held at 80 $\pm$ 5ºF and 50 $\pm$ 5 percent relative humidity. We assessed percent mortality after 1 and 7 days.

In the ovicide test we used the Allen track sprayer to spray the insecticide on *H. virescens* eggs. The test concentrations were obtained by diluting the formulated insecticides with the appropriate amount of water. The insecticide application was made using the sprayer which was calibrated to deliver 20 gallons/Acre. Eggs on cheesecloth were collected daily from adult oviposition containers. Cheesecloth were then clipped into small rectangles (2x2 inch) containing 40-50 eggs. We selected the rectangle stripes with the highest percentage of viable eggs (brown-ring stage) for use in bioassays. These strips, one for each treatment, were held cold (40ºF) overnight prior to testing. Immediately after spray application, each strip was removed from its cardboard holder and placed, still wet, in an 8 oz solo plastic cup. The cup was covered with a plastic lid. Cups were held for two days in a room at 80 $\pm$ 5ºF and 50 $\pm$ 5 percent relative humidity, then for another day at 72ºF and ambient relative humidity. Mortality data were recorded after three days.

The adulticidal bioassays were done by diluting the experimental compounds in 10% sucrose water solution to make series of doses. The sucrose solution alone was used for the untreated control. We poured ~25 ml of each dose onto ~1.15 gm of absorbent cotton batting in one ounce plastic cups. One cup was placed on a filter paper in the base of a 16 OZ wide-mouth glass jar. Five unfed, one day

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old, male or female moths were placed in each jar. The jars were covered with two layers of cheesecloth that were secured by rubber bands. The test was held in the laboratory at 72 °F. Results are recorded 1,2 and 3 DAT and adults that can not move in an upright position are considered dead.

Insecticides

We used Larvin® brand thiodicarb 3.2F, Sherpa® brand cypermethrin 10, Decis® brand deltamethrin 2.5 EC, technical Methomyl and Bolstar® brand sulprofos 6E in this study.

Insects

Our cooperators collected *H. virescens* pupae or eggs from cotton fields; then shipped them to our laboratory. We used these insects to establish the populations needed to conduct our bioassays. In 1995 we received samples from Snook, Texas; Bellemina & Pratville, Alabama; Drew County, Arkansas; Macon Ridge Research Station & Red River Research Station in Bossier City, Louisiana and Rocky Mount, North Carolina.

Results and discussion.

In this report we calculated resistance ratios by dividing the LC50 values of field populations by the LC50 value of the relatively susceptible field population collected from Rocky Mount, NC. The results of these comparisons provide more practical information than comparing the LC50 values of field populations to that of an inbred susceptible laboratory strain.

The resistance ratios presented in Table 1 show larvae collected from sites in the southeast and southwestern part of the cotton belt (Pratville & Bellemina, AL and Snook, TX) to have < 4 fold tolerance to Larvin. This low level of tolerance could be an indirect result of the successful boll weevil eradication program conducted in the southeast. This program eliminated the need for insecticide sprays against boll weevil and caused significant reduction in the overall insecticide selection pressure directed toward *Heliothis* spp. on cotton. Another reason for the low tolerance to Larvin could be attributed to the large untreated population that exists on wild and alternative host plants in the southeastern part of the belt. These untreated areas act as refugia and tend to dilute the effect of insecticide selection pressure over the local population (Taylor et al. 1995). The resistance management program implemented in the southwestern part of the belt provides a partial explanation for the low tolerance to Larvin shown by the larvae collected from Snook, TX.

Larvae obtained from sites in the mid-south (Arkansas and Louisiana) demonstrated <8 fold resistance to Larvin, the 11.6 fold found in Drew County, AR 3 in the middle of the growing season was the only exception. This relatively higher level of resistance could be due to the fact that farmers in the mid-south apply as many as 9 to 11 sprays on cotton to control *H. Virescens* larvae. This intense insecticide selection pressure will increase tolerance to insecticides and explain the difference in performance of Larvin against the larvae found in the southeast and those collected from the mid-south. Although larvae collected from sites in the mid-south demonstrated higher resistance to Larvin, the resistance ratios presented in Table 2 showed the eggs collected from Arkansas and Louisiana to have only 3.5 and 2.5 fold of tolerance to Larvin, respectively. Similar findings were reported by Gunning et al. (1992) in Australia. These researchers found Methomyl to be significantly more effective against eggs than larvae of *H. armigera*. This difference in toxicity could be related to the fact that detoxification mechanisms of the embryo are often not as well developed as in the larvae (Brattsten 1988). These findings raise the possibility that reduction in Larvin performance against larvae collected from sites in the mid-south could be offset by its good toxicity against eggs in this area.

Unlike Larvin, the fiducial limits of the two synthetic pyrethroid Sherpa and Decis listed in Table 3 and 4 did not overlap with the fiducial limits of the larvae obtained from Rocky Mount, NC. This indicates that the LC50 values of larvae obtained from various sites in the cotton belt were all significantly higher than the LC50 value of the susceptible larvae collected from Rocky Mount, NC. Also, resistance ratios show larvae obtained from southeastern and southwestern parts of the belt to have 5 to 15 fold resistance to Sherpa and 6 to 44.4 fold resistance to Decis (Tables 3 and 4). This indicates that despite the reduction in insecticide selection pressure and the implementation of resistance management programs, larvae in these areas continue to possess high levels of resistance to synthetic pyrethroids. In the mid-south resistance ratios ranged from 9 to 27 fold against Sherpa and 14 to 31 fold against Decis (Tables 3 and 4). In addition to poor activity against larvae Sherpa proved to be less effective than Larvin when tested against adults of *H. Virescens*. For example, the results presented in Table 5 show Sherpa to be 10.1 and >6.8 fold less effective than Larvin against males and 3.3 and >2.3 fold less effective against females collected from Rocky Mount and Drew County, respectively.

The above data indicate that high levels of resistance to synthetic pyrethroids in *H. virescens* larvae did not result in a high level of cross-resistance to Larvin (Figure 1). These data are in agreement with the findings of Gunning et al. (1991) that synthetic pyrethroid resistant populations of *Helicoverpa armigera* in Australia did not demonstrate cross-resistance to Methomyl, Larvin and the two

organophosphates Bolstar and Curacron. One reason for this lack of cross-resistance is the discovery that target site insensitivity is one of the major mechanisms responsible for resistance to synthetic pyrethroids in *H. Virescens* larvae (Gladwell et. al. 1990). Since the target site for carbamate and organophosphate insecticides is different from that of synthetic pyrethroids, it is not surprising that the high resistance to synthetic pyrethroids did not extend to insecticides that belong to these two classes.

In Rhône-Poulenc's workshop held in 1995 in Australia, R. V. Gunning (New South Wales Ag. & Fisheries, Australia) reported that resistance to Methomyl in *H. armigera* caused cross-resistance to Larvin. At first look these findings are not a total surprise since Larvin and Methomyl share the same mode of action. Also, Larvin molecular structure consists of two molecules of Methomyl connected together with a sulfur atom. However, these interesting results were in conflict with her earlier findings where she reported no cross-resistance between Larvin and Methomyl (Gunning et al. 1992). These conflicting results prompted us to include Methomyl in our 1995 monitoring program. Unlike Larvin, the lack of overlap of fiducial limits show Methomyl to be significantly less effective against larvae collected from sites in the mid-south (AR and LA), southeast (AL) and southwest (TX) than those collected from Rocky Mount, NC (Table 5). A comparison between the LC50 values presented in Table 6 show Larvin to be 2 to 32 fold more toxic than Methomyl. These results indicate that although Larvin and Methomyl share a number of biochemical and chemical characteristics, Methomyl proved to be significantly less effective than Larvin against *H. virescens* larvae. These results are in agreement with the findings of Mann et al. (1995). Their results showed Larvin to be more effective than Methomyl, Bolstar and Curacron for season long control of *Helicoverpa zea* and *Heliothis virescens* on cotton in South Carolina. The inferior biological activity of Methomyl could be due to its higher water solubility and/or shorter residual life relative to Larvin.

The organophosphate Bolstar is another cotton insecticide that has similar mode of action to Larvin (the two insecticides inhibit acetylcholine esterase enzyme). Relative to Larvin, Bolstar was 2 to 5 fold less effective against the larvae obtained from sites in the southeast (Table 7). These data are similar to the findings of Mann et al. (1995). However, Bolstar was about 2 fold more effective than Larvin against populations obtained from the mid-south. We found the two compounds to possess about the same level of toxicity to the insects obtained from the southwest.

Changes in the intensity of selection pressure during the cotton growing season could have a direct impact on the level of resistance to the insecticides used for *H. Virescens* control. We evaluated the toxicity of Larvin against larvae obtained from Drew County., AR at the beginning (AR 1), middle (AR 2&3) and end (AR 4) of the season. The lack of significant differences among the LC_{50} values of these collections, as indicated by the overlap of fiducial limits (Table 1), indicated that in 1995 Larvin provided consistent toxicity in Drew County throughout the cotton growing season. However, the resistance ratios obtained at different times from Drew County show low tolerance to Larvin (3.3 fold) at the beginning of the cotton growing season (Table 1). This low level of tolerance could be due to the light insecticide selection pressure at the beginning of the season. Another possibility was provided by Daly and Fisk (1995) who found adult of *Helicoverpa armigera* emerging from winter diapause early in the season to have lower level of resistance to insecticides. Resistance to Larvin increased in the middle (6.2 to 11.6 fold) then declined (6.8 fold) at the end of the season (Table 1). These data may reflect the gradual increase in the intensity of insecticide selection pressure toward the middle of the season. Susceptible insects entering cotton fields from nearby refugia at the end of the season could provide partial explanation for the decline in resistance.

In Figure 2, we summarized the performance history of Larvin from 1990 to 1995. We detected a gradual decline in the LC_{50} values for Larvin against larvae collected from Snook, TX. These data suggest that, in the last six years, resistance management programs in this area resulted in gradual improvement in the toxicity of Larvin. However, the LC50 value of Larvin in 1995 was higher than those generated in 1994 and 1993 from two sites in Louisiana. Also, the LC50 value we obtained in 1995 was higher than the one obtained from a site in Arkansas in 1994. This decrease in the larvicidal activity of Larvin may be the result of the intense insecticide selection pressure directed toward the unusually high populations of *H. Virescens* that invaded the mid-south in 1995.

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Table 1. 1995 Results of 7 DAT foliar spray tests showing the toxicity of Larvin 3.2F to *Heliothis virescens* larvae collected from different sites in the cotton belt.Foliar spray test.

	Foliar spray test	
Collection site	RR*	LC_{50}(ppm)
Drew County., AR 1	3.3	102.5 (63.6-165.3)**
Drew County., AR 2	6.2	193.2 (125.2-298.0)
Drew County., AR 3	11.6	358.6 (180.9-710.8)
Drew County., AR 4	6.8	211.9 (114.6-391.8)
Macon Ridge., LA	3.6	112.0
Bossier City, LA	7.6	236.0 (167-334)
Pratville, AL	0.5	16.0
Bellmina, AL	2.4	75.6 (34.4-166.4)
Snook, TX 1	2.6	82.1 (40.6-166)
Snook, TX 2	3.5	109.0 (72.1-164.6)
Rocky Mount, NC	31.0	(19.4-49.5)

* Resistance ratio = LC50 of field population / LC50 of Rocky Mount, NC

** 95% Fiducial limits.

Table 2. 1995 Ovicide (Black head) toxicity of Larvin to *H. virescens* collected from different sites in USA cotton belt.

	Foliar spray test	
Site	LC_{50}(ppm)	RR*
Pratville, AL	2237.5 (1873.2-2672.5)	1.2
Bellemina, AL	3326.4 (2711.5-4080.8)	1.9
Drew Co., AR 3	6161.2 (5727.3-6628.1)	3.5
Bossier City, LA	4368.9	2.5
Rocky Mount, NC	1776 (1304-2420)	-

* Resistance ratio = LC50 of field population / LC50 of Rocky Mount, NC

** 95% Fiducial limits.

Table 3. 1995 Results of 7 DAT foliar spray tests showing the toxicity of Sherpa 10 to *Heliothis virescens* larvae collected from different sites in the cotton belt.

	Foliar spray test	
Collection site	RR*	LC_{50}(ppm)
Drew CO. AR 1	9.1	143.8 (101.2-204.4)**
Drew CO., AR 3	22.5	355.0
Drew CO., AR 4	16.4	258.5 (165.4-403.8)
Macon Ridge., LA 1	15.8	250
Macon Ridge., LA 2	27.0	427
Bossier City, LA	20.1	317.1 (235-428)
Pratville, AL	4.9	78.0
Bellmina, AL	>15.8	>250(40%M)
Snook, TX 1	7.0	110.1 (51.0-238.0)
Snook, TX 2	14.6	230.2 (149.4-354.7)
Rocky Mount, NC	15.8	(5.2-48.4)

* Resistance ratio = LC50 of field population / LC50 of Rocky Mount, NC

** 95% Fiducial limits.

Table 4. 1995 Results of 7 DAT foliar spray tests showing the toxicity of Decis 2.5 to *Heliothis virescens* larvae collected from different sites in the cotton belt.

	Foliar spray test	
Collection site	RR*	LC_{50}(ppm)
Drew CO., AR 1	14.1	17.0 (10.9-26.4)**
Drew CO., AR 3	26.7	32
Drew CO., AR 4	26.5	31.8 (20.8-48-6)
Macon Rdige., LA 1	13.3	16
Macon Ridge, LA 2	31.7	38
Bossier City, LA	13.1	15.7 (6.7-36.5)
Pratville, AL	6.1	7.3
Bellmina, AL	>6.7	>8.0(20%M)
Snook, TX 2	44.4	53.3 (21.1-134.3)
Rocky Mount, NC		1.2 (0.6-2.7)

* Resistance ratio = LC50 of field population / LC50 of Rocky Mount, NC

** 95% Fiducial limits.

Table 5. Toxicity of insecticides to the adults of susceptible and resistant *H. virescens* collected from different sites in USA cotton belt.

Insecticides	LC_{50}(ppm)		
	Site	Males	Females
Larvin 3.2F	Rocky Mount, NC	0.7	~4.0
Sherpa 10	Rocky Mount, NC	~7.1	~13.1
Larvin 3.2F	Drew County, AR	<4.0	11.3
Sherpa 10	Drew County, AR	~27.0	>32.0

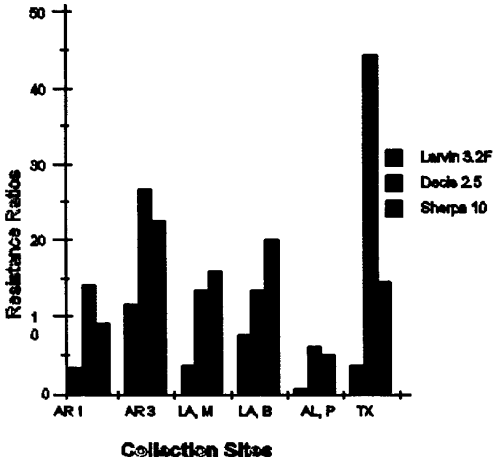

Figure 1. Comparison between the resistance ratios of Larvin, Decis and Sherpa.

Table 6. 1995 Results of 7 DAT foliar spray tests showing the toxicity of Methomyl (Technical) and Larvin 3.2F to *Heliothis virescens* larvae collected from different sites in the cotton belt.

Collection site	LC_{50}(ppm)		
	Methomyl	Larvin	Methomyl/Larvin
Drew CO., AR 1	381.2 (269-540)*	102.5 (64-165)	3.7
Drew CO., AR 2	>500.0	193.0	>2.6
Drew CO., AR 3	~1600.0	359.0	4.5
Drew CO., AR 4	667.1 (301.1478)	211.9 (115-392)	3.1
Macon Ridge, LA 1	310.0	112.0	2.8
Macon Ridge., LA 2	1708.0	500.0	3.4
Bossier City, LA	773.9 (516-1162)	236 (167-334)	3.3
Pratville, AL	505.0	16.0	31.6
Bellmina, AL	741.8 (299-1838)	75.6 (34-166)	9.8
Snook, TX 1	1212.0	82.1	14.8
Rocky Mount, NC	141.3 (96-209)	31.0 (19-50)	4.6

* 95% Fiducial limits.

Table 7. 1995 Results of 7 DAT foliar spray tests showing the toxicity of Bolstar 6E to *Heliothis virescens* larvae collected from different sites in the cotton belt.

Collection site	LC_{50}(ppm)		
	Bolstar	Larvin	Bolstar/Larvin
Drew CO., AR 1	108.5 (79-149)*	102.5 (64-165)	1.1
Drew CO., AR 2	32.0	193.0	0.2
Drew CO., AR 3	170.7 (121-241)	359.0 --	0.5
Drew CO., AR 4	230.1 (139-381)	211.9 (115-392)	1.1
Macon Ridge,. LA 1	86.0	112.0	0.8
Macon Ridge., LA 2	252.0	500.0	0.5
Bossier City, LA	116.9	236.0 (167-334)	0.5
Pratville, AL	86.0	16.0	5.4
Bellmina, AL	136.0 (92-201)	75.6 (34-166)	1.8
Snook, TX 1	99.5 (59-169)	82.1	1.2
Rocky Mount, NC	146.1 (96-223)	31.0 (19-50)	4.7

*95 Fiducial limits.

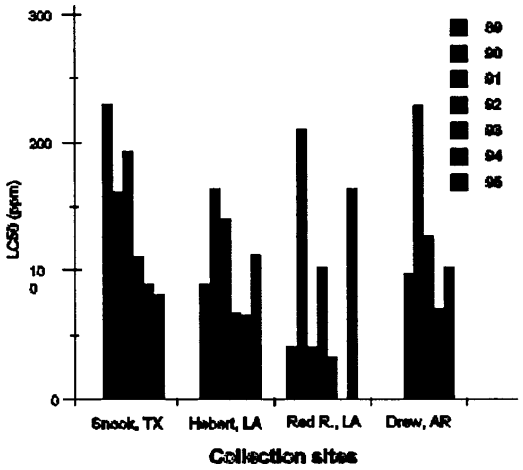

Figure 2. Historical performance of Larvin against *H. virescens* collected from four sites in the USA cotton belt.