2006 Beltwide Cotton Conferences, San Antonio, Texas - January 3 - 6, 2006

STATUS OF RESISTANCE IN TARNISHED PLANT BUG

Gordon Snodgrass
Stoneville, MS

Abstract

A survey was conducted in August and September 2005 to measure tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), and resistance to acephate with a glass-vial bioassay. Plant bug populations collected from wild hosts near cotton fields from twenty locations in the Delta region of Mississippi and ten locations in the hills region of Mississippi were tested. A population near Crossett, AR was used as a standard susceptible population to which collected populations were compared for relative resistance. Six populations from the Delta and four from the hills were found to have elevated resistance to acephate as indicated by three-fold or higher resistance ratios as compared to a susceptible population. Adults from a population found near Rolling Fork, MS that had 3.6-fold resistance to acephate were tested by caging them on cotton treated with commercial insecticides at labeled rates. Mortalities in treatments with acephate at 0.5 and 1.0 lbs AI/acre at 48 h after treatment were 39 and 48%, respectively. Mortalities in treatments with dicrotophos (0.5 lbs AI/acre), cyfluthrin (0.033 lbs AI/acre) and oxamyl (0.33 lbs AI/acre) were 44, 25, and 20%, respectively. These results showed that the plant bug population from Rolling Fork had resistance to three different classes of insecticides high enough to cause control failures in the field. Plant bug populations from all thirty sample locations will be tested in May and August-September of 2006 to again determine their insecticide resistance.

Introduction

Tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), are controlled in cotton exclusively with insecticides. Some pyrethroid insecticides were very effective for plant bug control in cotton during the 1980's. However, in 1993 plant bugs were found in cotton in the Delta of Mississippi that was highly resistant to pyrethroid insecticides with multiple resistance to some organophosphate and cyclodiene insecticides (Snodgrass 1996a). Pyrethroid resistance was found to be widespread in plant bug populations in the Delta of Arkansas, Louisiana, and Mississippi (Pankey et al. 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2000). This resistance changed each year with the most resistant populations found in the fall (after exposure of plant bugs to insecticides in cotton during the growing season) compared to the spring (Snodgrass and Scott 2000).

Resistance to pyrethroid and organophosphate insecticides has been shown to at least partly metabolic. Holloway et al. (1998) found that pyrethroid resistant plant bugs had elevated numbers and amounts of enzymes that could detoxify some insecticides. Zhu and Snodgrass (2003) found that pyrethroid resistance in plant bugs was due in part to elevated gene expression of cytochrome P450s caused by a cytochrome P450 gene mutation. Zhu et al (2004) found elevated esterase activity in a malathion resistant plant bug population. The underlying cause was thought to be increased esterase gene expression, rather than a mutation. Malathion resistant plant bugs were also found to have elevated levels of glutathione S-transferases (GSTs) similar to GSTs from other insects that conferred organophosphate resistance (Zhu et al. 2006). The underlying cause for resistance in tarnished plant bugs to other commonly used organophosphates such as acephate or dicrotophos has not been studied.

Insecticides recommended for tarnished plant bug control by the Cooperative Extension Service in the mid-south include the organophosphates acephate, dicrotophos, malathion, methamidophos, and profenofos; the carbamate oxamyl; the neonicotinoids imidacloprid and thiamethoxam; and the insect growth regulator novaluron (MS only). Acephate and dicrotophos are the most commonly used insecticides to control large populations in cotton with acephate being the insecticide most frequently used. Because of the importance of acephate in plant bug control and the threat of resistance development, resistance levels have been monitored in plant bug populations in the Delta each fall since 1998 (Snodgrass and Scott 2002). For the period of 1998 through 2000 most resistance levels at the 20 locations used in the study were 2-fold or less, and only a slight increase in tolerance to acephate over the three-year period was found. A similar pattern was found from 2001 through 2004 (G. L. S. unpublished data). An increase in resistance was found at several locations in 2005, and results from this year are presented in this manuscript.

Materials and Methods

Tarnished plant bugs were collected with a sweep net from wild hosts near cotton fields at twenty locations in the Delta (four in AR, two in LA, and fourteen in MS). These same locations were used each year from 1998 through 2005. Collections were made in August and September of each year. An additional ten locations in Mississippi were added in 2005. These locations were outside the Delta in the hill region of Mississippi where cotton is grown (Table 1).

Adults from each collection location were tested for resistance to acephate using a glass-vial bioassay developed by Snodgrass (1996b). In this bioassay, adults were placed into 20-ml glass liquid scintillation vials (two adults per vial) that were previously prepared by coating their inner surface with acephate. Acephate was applied to each vial by pipetting 0.5 ml of acephate diluted in acetone (pesticide grade, Fisher, Fair Lawn, NJ) into the vial. The vial was then rolled on a hotdog cooker (Star MFG, Smithville, TN). This process evaporated the acetone and left the insecticide as a residue on the inner surface of the vial. In all tests, the acephate was mixed with acetone and applied to the vials on the same day the test was performed. Technical grade acephate was purchased from Chem Service, West Chester, PA. Prior to adding the adults to a vial for testing, a piece of green bean was added to the vial for food. Green beans were surface sterilized (see Snodgrass 1996b) and cut transversely into pieces about 3-mm thick and one piece was used in each vial. After plant bugs were placed in a vial, a cotton ball was placed in the vial opening to confine the bugs. At least five doses were tested, and each test was replicated three times. Each replication had five vials, each containing two adults, and mortality was determined after 24 h of exposure. Control vials were treated only with acetone, and control mortality was rare and never >3.3%. Data were corrected for control mortality using Abbott's (1925) formula before analysis. Data from the glass-vial bioassays were analyzed assuming the probit model (Proc Probit: SAS Institute 1999). LC_{50} values for each location were compared to the LC_{50} for acephate for susceptible bugs to obtain a resistance ratio (RR). The susceptible bugs were collected near Crossett, AR where no row crops are grown.

Insecticide resistance was studied in a field test conducted on 1-3 September 2005 using cotton ('Suregrow 215') located on the Delta Branch Experiment Station, Mississippi State University, Stoneville, MS. Plots were four rows by 40 ft in length. The plots were treated using a high clearance sprayer calibrated to deliver 9.5 gal/acre at 40 psi. Treatments included an untreated check, acephate (Orthene®) 0.50 lbs AI/acre, acephate 1.0 lbs AI/acre, dicrotophos (Bidrin®) 0.5 lbs AI/acre, cyfluthrin (Bathyroid®) 0.033 lbs AI/acre, and oxamyl (Vydate®) 0.33 lbs AI/acre. There were four replications of each treatment arranged in a randomized complete block design. In each replication, ten sleeve cages were used to confine two adult plant bugs in each cage on the mainstem terminal of a plant. Cages were placed on the plants after the treatment applications had dried. Adult plant bugs used in the test were collected from pigweed, *Amaranthus spp.*, near Rolling Fork, MS. These plant bugs had been determined to have 3.6-fold resistance to acephate in the glass-vial bioassay. A second field test was conducted on 8-10 September using adults collected from Rolling Fork and a second population of adults collected from pigweed near Indianola, MS. The population from Indianola had 1.6-fold resistance to acephate. The second test was identical to the first test except the treatments used included an untreated check and two rates of acephate (Orthene®) 0.50 and 1.0 lbs AI/acre replicated three times. Mortality in the two acephate treatments was compared between the two populations of plant bugs using PROC MIXED (SAS Institute 1999).

Results and Discussion

Results from the resistance survey conducted in 2005 showed that plant bug populations at six of the twenty Delta locations had LC_{50} values high enough to produce three-fold or higher levels of resistance (RR of 3 or >) to acephate (Table 1). Three-fold or higher resistance to acephate was also found in plant bug populations from four of the ten locations tested from the hills region. During the previous five years of testing plant bug populations from three locations in the Delta were found to have three-fold or higher resistance to acephate.

The first field test was conducted to determine how a plant bug population with at least three-fold resistance to acephate (as determined with the glass-vial bioassay) responded under field conditions to treatments made for control with commercial insecticides at recommended rates. The plant bug population from Rolling Fork used in the test had 3.6-fold resistance to acephate, and as shown in Table 2, none of the insecticides tested provided acceptable control. The highest mortalities were obtained with the high rate of acephate (1.0 lbs AI/acre) and dicrotophos, and were 48 and 44%, respectively. Mortalities for cyfluthrin and oxamyl were only 25 and 20%, respectively. Field conditions for the test were more favorable for control of the plant bugs than those normally encountered. The plant

bugs were caged on the main stem terminals and spray coverage of the terminals is usually better than the coverage obtained lower in the plant canopy where plant bugs are also commonly found. Even under the optimal conditions for control, none of the insecticides tested, which included three classes of insecticides, provided adequate control of the plant bug population from Rolling Fork.

The second field test again used adults from Rolling Fork, and for comparison, adults collected from near Indianola, MS. The plant bugs from Indianola had about half of the resistance to acephate (1.6-fold) that the plant bugs from Rolling Fork had (3.6-fold). The plant bugs from Indianola were significantly more susceptible to acephate than those from Rolling Fork at both field rates tested (Table 3). The 0.5 lbs AI/acre rate of acephate killed 62% of the plant bugs from Indianola as compared to 32% of the plant bugs from Rolling Fork. A mortality of 62% is close to the average mortality of 66% for plant bugs tested with 0.5 lbs AI/acre reported by Snodgrass and Scott (2002) who summarized data from eight field studies in the mid-South conducted from 1997 through 1999. The high rate (1.0 lbs AI/acre) of acephate killed 90% of the plant bugs from Indianola and 63% of the plant bugs from Rolling Fork. This was an increase in mortality for the plant bugs from Rolling Fork over the mortality (48%) found with the 1.0 lbs AI/acre rate in the first test (Table 2). However, the 1.0 lbs AI/acre rate is not a recommended rate of acephate for plant bug control in cotton.

Results from the two field tests indicated that tarnished plant bug populations with three-fold or higher resistance to acephate would be very difficult to control with organophosphate, pyrethroid, and carbamate insecticides. Only one of the ten populations found with this level of resistance to acephate was tested, and results from testing other resistant populations could vary. However, the finding that ten of the thirty populations tested might be as difficult to control as the population from Rolling Fork is cause for concern. It could be that resistance to acephate is recessive and the expression of the resistance will decline as new generations are produced on wild hosts where no selection with insecticides occurs. This was found with pyrethroid resistance in plant bug populations in the mid-South (Snodgrass 1996a, Snodgrass and Scott 2000). However, selection is likely to occur more rapidly in subsequent years as the level of resistant alleles to acephate increases. Resistance monitoring will again be conducted in May of 2006 at the same locations used in 2005. By May, plant bug populations will have gone through at least three new generations on wild hosts. Hopefully, resistance levels will decline before plant bugs move into cotton in June and July. An insecticide resistance management plan may be needed for the mid-South to slow resistance development to the organophosphates and delay resistance development to the neonicotenoids.

References Cited

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

Hollingsworth, R. G., D.C. Steinkraus, and N. P. Tugwell. 1997. Response of Arkansas populations of tarnished plant bugs (Heteroptera: Miridae) to insecticides, and tolerance differences between nymphs and adults. J. Econ. Entomol. 90: 21-26.

Holloway, J. W., B. R. Leonard, J. A. Ottea, J. H. Pankey, and J. B. Graves. 1998. Insecticide resistance and synergism of pyrethroid toxicity in the tarnished plant bug, *Lygus lineolaris*. Proc. Beltwide Cotton Conference, pp. 947-949. National Cotton Council of America, Memphis, TN.

Pankey, J. H., B. R. Leonard, J. B. Graves, and E. Burris. 1996. Toxicity of acephate, cypermethrin, and oxamyl to tarnished plant bugs in vial bioassays and cage studies on cotton, pp. 882-887. In Proc. Beltwide Cotton Production Research Conference, Nashville, TN. National Cotton Council, Memphis, TN.

SAS Institute. 1999. SAS/STAT user's guide, version 8. SAS Institute, Cary, NC.

Snodgrass, G. L. 1996a. Pyrethroid resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol. 89: 783-790.

Snodgrass, G. L. 1996b. Glass-vial bioassay to estimate insecticide resistance in adult tarnished plant bugs (Heteroptera: Miridae). J. Econ. Entomol. 89: 1053-1059.

Snodgrass, G. L. and W. P. Scott. 2000. Seasonal changes in pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations during a three-year period in the delta area of Arkansas, Louisiana, and Mississippi. J. Econ. Entomol. 93: 441-446.

Snodgrass. G. L. and W. P. Scott. 2002. Tolerance to acephate in tarnished plant bug (Heteroptera: Miridae) populations in the Mississippi River Delta. Southwest. Entomol. 27: 191-199.

Zhu, Y. C. and G. L. Snodgrass. 2003. Cytochrome P450 CYP6 x 1 cDNAs and mRNA expression levels in three strains of the tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae) having different susceptibilities to pyrethroid insecticides. Insect Mol. Biol. 12: 39-49.

Zhu, Y. C., G. L. Snodgrass, and M. S. Chen. 2004. Enhanced esterase gene expression and activity in a malathion-resistant strain of the tarnished plant bug, *Lygus lineolaris*. Insect Biochem. Molec. Biol. 34: 1175-1186.

Zhu, Y. C., G. L. Snodgrass, and M. S. Chen. 2006. Comparative study on glutathione s-transferase activity, cDNA, and gene expression between malathion susceptible and resistant strains of the tarnished plant bug, *Lygus lineolaris*. Insect Biochem. Molec. Biol. (submitted Dec. 2005).

Table 1. Mortality of adult tarnished plant bugs from twenty locations in the Mississippi River Delta and ten locations in the hills of Mississippi exposed to acephate in a glass-vial bioassay in 2005.

Location	N	Slope ± SE	LC_{50}[a]	95% CL	X^2	$P>X^2$	RR_{50}[b]
			Mississippi Delta				
Avon	210	1.5 ± 0.20	14.4	12.2-16.9	9.4	0.90	4.6
Greenville	210	1.0 ± 0.13	12.0	9.6-15.1	14.2	0.59	3.9
Rolling Fork	180	1.2 ± 0.18	11.1	9.1-13.8	8.3	0.82	3.6
Clarksdale	180	1.3 ± 0.18	9.4	7.8-11.1	9.2	0.76	3.0
Minter City	180	1.1 ± 0.16	7.7	6.1-9.5	11.7	0.59	2.5
Ruleville	150	0.9 ± 0.19	6.4	4.8-8.5	5.7	0.84	2.1
Marks	150	1.7 ± 0.25	6.2	5.1-7.3	5.9	0.82	2.0
Tunica	150	1.5 ± 0.23	5.4	4.4-6.4	8.4	0.59	1.7
Indianola	150	0.9 ± 0.15	4.9	3.4-6.4	2.4	0.99	1.6
Vicksburg	150	1.2 ± 0.21	4.5	3.4-5.5	5.1	0.88	1.4
Indianola[c]	150	1.0 ± 0.21	3.5	2.3-4.5	5.7	0.84	1.1
Winterville	150	1.4 ± 0.25	3.5	2.6-4.3	5.9	0.82	1.1
Thornton	150	0.9 ± 0.20	3.2	1.8-4.3	5.3	0.87	1.0
Greenwood	150	1.5 ± 0.28	3.1	2.3-3.8	4.1	0.94	1.0
			Mississippi Hills				
Bruce	210	1.1 ± 0.15	9.9	8.1-12.0	16.9	0.39	3.2
Winona	180	1.7 ± 0.23	9.8	8.4-11.5	9.4	0.74	3.2
Elliott	180	1.4 ± 0.20	9.6	8.0-11.5	19.6	0.11	3.1
Gore Springs	180	1.0 ± 0.17	9.2	7.3-11.6	7.2	0.89	3.0
Bradford	180	1.3 ± 0.21	6.9	5.8-8.3	5.8	0.95	2.2
Oxford	180	1.0 ± 0.16	6.6	5.0-8.3	6.9	0.91	2.1
Charleston	150	1.0 ± 0.20	4.5	3.2-5.8	7.8	0.65	1.5
Water Valley	150	1.2 ± 0.21	4.2	3.1-5.2	7.7	0.66	1.3
Batesville	150	1.3 ± 0.22	3.9	2.9-4.8	4.5	0.92	1.2
			Louisiana Delta				
Lake Providence	210	1.4 ± 0.18	0.7	9.1-12.6	13.6	0.63	3.5
Transylvania	150	1.1 ± 0.17	10.0	8.1-12.4	12.3	0.50	3.2
			Arkansas Delta				
Gould	150	1.1 ± 0.17	6.2	4.9-7.7	10.6	0.64	2.0
Grady	150	1.7 ± 0.21	4.6	3.8-5.4	4.1	0.95	1.5
Parkdale	150	1.1 ± 0.20	4.4	3.3-5.5	7.9	0.64	1.4
Lake Village	150	1.0 ± 0.17	3.8	2.5-5.0	5.0	0.97	1.2
Crossett[d]	270	1.8 ± 0.22	3.1	2.6-3.6	5.80	0.20	----

[a] Acephate concentrations are micrograms per vial; survival was scored at 24 h.

[b] RR_{50} = resistance ratio. LC_{50} of test location divided by LC_{50} for a susceptible population from Crossett, AR (3.1 ug).

[c] Two locations near Indianola were used. The first location was south of Indianola along U. S. Highway 49, the second location was west of Indianola along U. S. Highway 82.

[d] The Crossett, AR population was from an area not located near row crops and was used as the standard basis for comparison to determine resistance ratios. This population was also used in Snodgrass and Scott (2002) to evaluate resistance to acephate.

Table 2. Mortality of adult tarnished plant bugs from Rolling Fork, Mississippi with 3.6-fold resistance to acephate caged for 48 h on cotton plants treated with different insecticides.

Treatment (lbs AI/acre)		Mean % mortality[a] (± SE)
Untreated check		8 (± 1.4)
Acephate (Orthene®)	(0.50)	39 (± 4.9)
Acephate	(1.00)	48 (± 8.9)
Dicrotophos (Bidrin®)	(0.50)	44 (± 11.3)
Cyfluthrin (Baythroid®)	(0.033)	25 (± 5.8)
Oxamyl (Vydate®)	(0.33)	20 (± 1.2)

[a] The mean is for four replications with ten cages with two adults per cage per replication.

Table 3. Mortality of adult plant bugs from Rolling Fork and Indianola, Mississippi caged on cotton treated with acephate at 48 h after treatment. The plant bugs from Rolling Fork had 3.6-fold resistance to acephate, those from Indianola had 1.6-fold resistance.

Treatment (lbs AI/acre)		Mean % mortality[a]			
		Rolling Fork	Indianola	**F**[b]	*P*>**F**
Untreated check		5	7	0.10	0.77
Acephate (Orthene®)	(0.50)	32	62	39.9	0.003
Acephate	(1.00)	63	90	20.9	0.01

[a] The means are for three replications with ten cages with two adults per cage per replication.
[b] The F values and probabilities are for comparison between mortality in the plant bugs from Rolling Fork with mortality in the plant bugs from Indianola for each of the three treatments. The degrees of freedom in each comparison are 1 and 4.