# SEASONAL CHANGES IN PYRETHROID RESISTANCE IN TARNISHED PLANT BUG POPULATIONS IN THE MISSISSIPPI DELTA

**G. L. Snodgrass and W. P. Scott**
**Research Entomologists, USDA-ARS**
**Southern Insect Management Laboratory**
**Stoneville, MS**

## Abstract

A discriminating dose bioassay was developed and used to determine pyrethroid resistance in tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), populations in the Mississippi River Delta of Arkansas, Louisiana, and Mississippi. Adults from 71 collection locations in the spring and from 72 locations in the fall (mostly the same locations used in the spring) were tested with the bioassay for pyrethroid resistance. Mortality of <90% among a minimum of 50 adult plant bugs per test population exposed for 3 h in 20-ml glass liquid scintillation vials treated with 15 $\mu$g of permethrin was defined as pyrethroid resistance. In the spring, 57.7% (41) of the collection locations had plant bug populations with pyrethroid resistance, this increased to 84.7% (61) in the fall. These results showed that pyrethroid resistance is widespread in plant bug populations in the Delta. Plant bugs in the Delta can produce three to four generations on weeds during September-November after the cotton growing season over, and in April-May of the following year. This allows resistance in these populations to decline in the absence of insecticide selection pressure. However, pyrethroids should not be used in cotton for plant bug control in the Delta in May and June, since over half of the populations tested in the spring had pyrethroid resistance.

## Introduction

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is a key early season pest of cotton in the southeastern United States. Its control in cotton is obtained almost exclusively by insecticides, including those found in the carbamate, organophosphate, and pyrethroid classes. The use of pyrethroid insecticides for plant bug control in early season has declined because insecticide resistance in the tobacco budworm, *Heliothis virescens* (F.). This has led to the use of a resistance management program in the Mississippi Delta (Anonymous 1986), in which pyrethroid use in cotton is delayed until July. Snodgrass (1994) and Snodgrass and Elzen (1995) reported on plant bug populations found in cotton in the Mississippi Delta in July of 1993 and 1994 which were highly resistant to pyrethroids. The extent of pyrethroid resistance in plant bug populations in the Delta has not been known. The present study provides this information along with information on how pyrethroid resistance changes in these populations during the year.

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## Materials and Methods

### Collection Locations

Tarnished plant bugs were collected from wild host plants for resistance testing with the discriminating dose bioassay using a sweep net. Bugs were aspirated from the sweep net after collection in the field and placed into paper ice-cream cartons (0.95 liter) with fresh green beans. The bugs were held in the containers for a 24-h period prior to being tested to allow any bugs injured during collection to die. At least 50 adults from each collection location were tested for pyrethroid resistance using a discriminating dose of 15 $\mu$g of permethrin per vial with an exposure time of three h. Fifty or more adults from each collection location were tested since Roush and Miller (1986) stated that a sample of 50 individuals would document resistance present at a frequency of $\geq$10% with 95% accuracy. In the spring (25 April-31 May, 1995) bugs from 71 locations (18 in AR, 3 in LA, and 50 in MS) were tested. Many of these same locations were sampled during the fall (7 September-5 October) when bugs from 72 locations (18 in AR, 6 in LA, and 48 in MS) were tested for pyrethroid resistance. In the fall survey, plant bug populations were tested after collection at the same 18 locations used in the spring in AR. Plant bugs were collected for testing in the fall at the same three locations used in the spring in LA, and an additional three locations were also used. All locations at which bugs were collected for testing in the fall in MS were the same ones used in the spring, except that two locations from the spring survey were dropped.

Collection locations were areas near fields or ditches along highways in the Delta which contained wild host plants. Locations were spaced at least 5 miles apart along a highway and locations were selected in the spring and were judged as areas likely to also have wild host plants in the fall. Highways used in the Delta included U. S. Highways 65, 82, and 165 in AR; 65 in LA; and 49, 61 and 82 in MS. State highways were Highway 1 in AR, and 1, 3, 6, 7, and 14 in MS. Collection locations along U.S. Highway 65 were located from near Pine Bluff, AR to Tallulah, LA; from Lake Village, AR to Greenwood, MS along U.S. Highway 82; from Tunica to Vicksburg, MS along U.S. Highway 61; from Parkdale to McGhee, AR along U.S. Highway 165; and from Greenwood to Yazoo City, MS along U.S. Highway 49. Collection locations were along smaller sections of the state highways listed previously.

### Bioassays

Two types of glass vial bioassays were used in the study. One type described by Snodgrass (1994) was used to determine plant bug mortality after 24 h of exposure to different rates of an insecticide in 20-ml glass liquid scintillation vials. Mortality at the different insecticide

rates was then used to calculate an $LC_{50}$ value for the insecticide. The second bioassay was a discriminating dose bioassay developed for use in the study reported here. To develop the discriminating dose bioassay, adult plant bugs from pyrethroid resistant and susceptible laboratory colonies maintained at the Southern Insect Management Laboratory, USDA-ARS, Stoneville, MS, were placed in glass liquid scintillation vials (20 ml) that had been treated with 10, 15, or 20 $\mu$g of permethrin. Technical grade permethrin (94.7% purity; FMC Corporation, Princeton, NJ) dissolved in acetone was coated on the inner surface of the vials using the procedure of Plapp and Vinson (1977). Two adults were placed into each vial and confined by placing a cotton ball in the vial opening. Five replications were used for each of the three concentrations of permethrin tested. In each replication were 10 permethrin treated vials and 10 acetone treated vials (untreated check). Adults were placed in the vials and mortality was recorded at 0.5 h intervals for three h. Adults were considered dead when they could no longer move or were unable to right themselves. The test was designed to determine a rate of permethrin and exposure time that would produce high (>90%) mortality in the susceptible bugs and low (<10%) mortality in the resistant bugs. This procedure followed the reasoning of McCutchen et al. (1989) and Kanga et al. (1993) in determining a discrimin-ating dose for tobacco budworms in adult vial tests.

**Spray Chamber Tests**

Four colonies of bugs were tested to determine how pyrethroid resistance levels in the populations, as determined with the discriminating dose bioassay, related to mortality obtained with a formulated pyrethroid insecticide under conditions that simulated those found in the field. Adults were placed in cages with cotton terminals after the terminals were treated using a spray chamber as described by Elzen et al. (1992). The spray chamber was calibrated to deliver 56 liters/ha at 2109 g/cm$^2$ with one hollow-cone nozzle (TX, Spraying Systems, Hammond, LA) at 3.2 km/h. The spray nozzle was positioned 30.5 cm above the spray surface. Ventillated paper cups (0.295 liter) were used as cages, and mortality was determined 24 h after placement in the cups. Ten cages (two adults per cage) were used in each replication. Controls were adults caged (two adults per cage) on cotton terminals treated with water. In both tests permethrin [Pounce 3.2 (EC), FMC Corp., Philadelphia, PA] at 0.11 kg AI/ha was used to treat the cotton terminals. In the first test (28 July), hybrid offspring obtained by crossing the pyrethroid resistant and susceptible laboratory colonies were tested as adults, along with adults from the resistant and susceptible colonies. In the second test (20 September), adults collected along U.S. Highway 65 near its junction with U.S. Highway 165 were tested along with adults from the susceptible laboratory colony. Data from both spray chamber bioassays were analyzed using analysis of variance (SAS Institute 1989) and means were separated using least significant difference (LSD) (P = 0.05). Treatment mortality was corrected based on mortality in the control groups using Abbot's (1925) formula before data were analyzed.

## Results and Discussion

**Bioassays**

The best separation in mortality between the susceptible and resistant plant bugs was obtained with 15 $\mu$g/vial of permethrin (Table 1). Differences in mortality between the resistant and susceptible plant bugs at this rate with 1.5 to 3 h of exposure were 85-86%, and during this time period less than 10% of the resistant bugs and >90% of the susceptible bugs were killed. Mortality in the susceptible colony was too low (a maximum of 80%) at the 10 $\mu$g/vial rate, while mortality was too high in the resistant bugs (14-28%) from 1.5 to 3.0 h of exposure using the 20 $\mu$g/vial rate of permethrin. Based on these results a rate of 15 $\mu$g/vial of permethrin and an exposure time of 3 h was used to test for pyrethroid resistance in the discriminating dose bioassay. Resistant populations were those in which mortality in the bioassay was <90%. The choice of the 3 h exposure period was arbitrary, since the test results showed that exposure periods of 1.5, 2.0, and 2.5 h would have worked as well.

**Spray Chamber Tests**

The hybrid adult plant bugs in test 1 were pyrethroid resistant since their mortality was 64% in the discriminating dose bioassay, and their $LC_{50}$ value for permethrin was 11.1 $\mu$g/vial (Table 2). Mortality from caging hybrid adults on cotton terminals treated with formulated permethrin in the spray chamber was only 3.6% which was significantly lower ($F = 8.36$; df = 2,4; $P>F = 0.04$) than the 41.9% obtained in the test with the susceptible colony. In the second test, the pyrethroid resistant adults collected from weeds in AR had a mortality of 78% in the discriminating dose bioassay and an $LC_{50}$ of 9.6 $\mu$g/vial of permethrin. Their mortality when caged on cotton terminal treated in the spray chamber was 8.7% which was significantly lower ($F = 25.29$; df = 1, 2; $P>F = 0.04$) than the 50.8% mortality obtained in the test with the susceptible colony. These results showed that the discriminating dose bioassay does detect pyrethroid resistant populations, and that mortality in the pyrethroid resistant populations was significantly lower than mortality in susceptible populations when exposed to a formulated pyrethroid insectide under simulated field conditions.

**Collection Locations**

In the spring survey, tarnished plant bug populations found at 30 of the 71 collection locations (42.3%) were susceptible to pyrethroids, while populations at 41 locations (57.7%) had pyrethroid resistance (Table 3). Plant bug populations with pyrethroid resistance tested in the fall had a large increase in numbers of populations. Only 11 locations (15.3%) had susceptible populations, while 61 locations (84.7%) had pyrethroid resistant populations.

The amount of resistance also increased since 13 locations (18%) were highly resistant having populations whose mortalities were <30% in the discriminating dose bioassay. The most resistant population in the spring survey had a mortality of 45.5% in the bioassay, while mortality was 2% in the most resistant population in the fall survey, and 27 locations had populations with mortalities less than 45.5% in the fall. The big increase in pyrethroid resistance was probably due to the exposure of the plant bugs to pyrethroid insecticides in cotton fields during the growing season.

Results of all tests show that pyrethroid resistance in tarnished plant bug pop-ulations can be monitored using the discriminating dose bioassay developed for use in this study. Pyrethroid resistance was found to be widespread in all three Delta states in which it was monitored. Pyrethroid resistance in plant bug populations was lowest in the spring then increased dramatically in the fall. The finding that 57.7% of the collection locations had plant bug popula-tions with pyrethroid resistance in the spring prior to the movement of the adult bugs into cotton in June and July is cause for concern. Pyrethroid resis-tant plant bugs are difficult to control in cotton since they are cross-resistant to all pyrethroids and have multiple resistance to other insecticides (Snodgrass and Elzen 1995). Pyrethroid use in cotton should be avoided during May and June to avoid increasing resistance problems in plant bugs.

Resistance in plant bugs to pyrethroids will be monitored again in the Delta in the spring and fall of 1996. Pyrethroid resistance in the plant bug popula-tions should decline prior to the spring survey in 1996, since by then they will have produced three to four generations on weeds without insecticide selection pressure. However, results from the fall of 1995 show that pyrethroid resistance in plant bugs is so widespread that no significant decline in resistance is expected.

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Table 1. Mortality in time of pyrethroid resistant and susceptible tarnished plant bugs exposed in 20-ml glass vials to three rates of permethrin.

| | | % Mortality[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | Rate | Time (h) | | | | | |
| Colony | permethrin ($\mu$g/vial) | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| Resistant | 10 | 0 | 0 | 2 | 2 | 2 | 2 |
| Susceptible | 10 | 6 | 45 | 56 | 69 | 79 | 80 |
| Resistant | 15 | 0 | 1 | 5 | 7 | 7 | 9 |
| Susceptible | 15 | 37 | 86 | 91 | 92 | 93 | 95 |
| Resistant | 20 | 2 | 9 | 14 | 23 | 28 | 28 |
| Susceptible | 20 | 71 | 91 | 93 | 94 | 95 | 95 |

[a] Mortality at each rate is based on 5 replications with 10 permethrin treated vials and 10 vials treated only with acetone (check) per replication. Two adults were used per vial, and check mortality in all replications of all rates was 0.

Table 2. Mortality of adult tarnished plant bugs caged (24 h) on cotton terminals treated in a spray chamber with Pounce (permethrin). Different pyrethroid resistance levels in the colonies are shown as $LC_{50}$ values for permethrin (determined in a glass vial bioassay), and as mortality in a discriminating dose bioassay.

| Colony[a] | N | $LC_{50}$ ($\mu$g/vial) | (95% CL) | Slope±SE | $\chi^2$ | % Mortality[b] discriminating dose | % Mortality[c] spray chamber |
|---|---|---|---|---|---|---|---|
| Test 1 | | | | | | | |
| Hybrid | 240 | 11.1 | (9.5-13.0) | 1.42±0.17 | 5.4 | 64 | 3.6b |
| Susceptible | 210 | 4.6 | (3.8-5.4) | 1.46±0.19 | 2.1 | 92 | 41.9a |
| Resistant | 300 | 52.1 | (44.9-60.4) | 1.25±0.13 | 8.6 | 09 | 0.0b |
| Test 2 | | | | | | | |
| AR | 240 | 9.6 | (8.1-11.3) | 1.25±0.16 | 2.3 | 78 | 8.7b |
| Susceptible | 180 | 6.2 | (4.9-7.7) | 1.11±0.17 | 3.1 | 92 | 50.8a |

In each test means in a column not followed by a common letter are significantly different (LSD, $P \leq 0.05$).

[a] The susceptible and resistant colonies are laboratory colonies (see text). The hybrid colony is the offspring from crossing the resistant and susceptible colonies, and the AR colony was collected from wild hosts near the junction of U.S. Highways 65 and 165 in AR.

[b] Mortality based on 100 adults of each colony exposed for three h (two adults per vial) in vials treated with 15 $\mu$g of permethrin per vial.

[c] Mortality corrected for check mortality using Abbott's (1925) formula. Percent mortality is the mean for three replications with 20 adults per replication caged on cotton terminals (two adults per cage) treated with Pounce (0.11 kg AI/ha).

Table 3. Collection locations in the Mississippi River Delta of Arkansas, Louisiana, and Mississippi with pyrethroid resistant and susceptible tarnished plant bug populations. Adults collected on wild host plants at each location were tested for resistance with a discriminating dose of 15 $\mu$g of permethrin.

| | Number of collection locations | | | | |
|---|---|---|---|---|---|
| | Spring[a] | | | | |
| | AR | LA | MS | Sum | % total |
| Susceptible[b] | 10 | 1 | 19 | 30 | 42.3 |
| Resistant | 8 | 2 | 31 | 41 | 57.7 |
| Highly resistant[b] | 0 | 0 | 0 | 0 | 0.0 |
| Sum | 18 | 3 | 50 | | |
| Total | | | | 71 | |
| | Spring[a] | | | | |
| | AR | LA | MS | Sum | % total |
| Susceptible[b] | 4 | 0 | 7 | 11 | 15.3 |
| Resistant | 12 | 2 | 34 | 48 | 66.7 |
| Highly resistant[b] | 2 | 4 | 7 | 13 | 18.0 |
| Sum | 18 | 6 | 48 | | |
| Total | | | | 72 | |

[a] Spring = 25 April-31 May, 1995; Fall = 7 September-5 October, 1995.

[b] Susceptible populations had 90% or greater mortality in the discriminating dose bioassay. Highly resistant populations had mortalities <30% in the bioassay.