

## Glass-Vial Bioassay To Estimate Insecticide Resistance in Adult Tarnished Plant Bugs (Heteroptera: Miridae)

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**ABSTRACT** A glass-vial bioassay was developed for use in estimating resistance to insecticides in adult tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois). When laboratory-reared insects of known age are used, the bioassay procedure requires that adults are not older than 10 d. Testing each sex separately was not required, and each glass-vial (treated on its inside with an insecticide) received a piece of green bean, *Phaseolus vulgaris* L., as food for the adults tested. Two or 3 adults were placed in each vial; mortality was determined after 24 h of exposure in the vial. The bioassay was used to determine insecticide resistance in 3 groups of tarnished plant bugs to 10 different insecticides. Significant differences in resistance among adults were found from the 3 groups with most of the insecticides. Data obtained with this glass-vial bioassay can be compared with data obtained from bioassays that use plant bugs collected from cotton, *Gossypium hirsutum* L., fields in which control with insecticides has been inadequate.

**KEY WORDS** *Lygus lineolaris*, insecticide resistance, glass-vial bioassay

THE TARNISHED PLANT bug, *Lygus lineolaris* (Palisot de Beauvois), is a major economic pest of cotton, *Gossypium hirsutum* L., in the southeastern United States. Tarnished plant bugs can damage presquaring cotton, causing growth deformities and a delay in fruiting and boll maturity (Hanny et al. 1977). This species also damages developing squares and causes delayed boll maturity and significant yield loss (Scott et al. 1986). Control of plant bugs in cotton is almost exclusively by use of insecticides. In the Mississippi Delta, for example, several organophosphates, pyrethroids, and 1 carbamate are commonly used (Layton 1994).

Use of pyrethroid insecticides for early-season cotton insect control has declined in the Delta because tobacco budworms, *Heliothis virescens* (F.), have become resistant to these insecticides. Resistance in this species led to the use of a resistance management program in the Mississippi Delta (Anonymous 1986), in which use of pyrethroids against tobacco budworms is delayed until July. During July and August, applications of pyrethroids for tobacco budworms also have controlled plant bugs in the treated fields. However, Snodgrass (1994) determined that tarnished plant bugs collected from a cotton field in the Mississippi Delta were very resistant to the pyrethroids bifenthrin and permethrin during July and August 1993. Although the frequency of this resistance in the Delta population is unknown, tolerance to the pyrethroids may become a big problem in controlling plant bugs. Difficulty in the control of plant bugs has also created a need for a rapid method of testing plant bugs for insecticide resistance.

Insecticide resistance in plant bugs has been studied with topical application of insecticides and with several different glass-vial bioassays. Cleveland and Furr (1979) and Cleveland (1985) used topical applications of insecticides to adults; they found increased resistance to some organophosphates in tarnished plant bugs collected in the Mississippi Delta. Brindley et al. (1982) used 8.9-g glass-vials with insecticide coated on their inner surface and 3 small alfalfa leaflets to enhance control survival to study resistance in *Lygus hesperus* Knight. They used 5 bugs per vial, held the vials in a portable incubator, and determined mortality after 16 h of exposure. Snodgrass and Scott (1988) modified the adult vial test developed by Plapp et al. (1987) to study resistance in bollworms, *Helicoverpa zea* (Boddie), and tobacco budworms, and used it to determine resistance to acephate and dimethoate in tarnished plant bugs collected in the Mississippi Delta. By their test procedure, a small piece of green bean, *Phaseolus vulgaris* L., was added to each vial to improve survival, 3 adults were tested per vial, and mortality was determined after 24 h of exposure in the vials. Knabe and Staetz (1991) also adapted the adult vial test of Plapp et al. (1987) to study resistance to pyrethroids in *L. hesperus*. They used 5 insects per vial with no food and determined mortality after 3 h of exposure.

Glass-vial bioassays are not as precise as topical application of a known amount of insecticide to each insect tested. However, glass-vial bioassays require inexpensive equipment and can be used by

growers, crop consultants, and researchers. Factors such as the number of bugs used per vial, presence or absence of food, and the length of time the bugs are exposed in the vials may affect the results of a glass-vial bioassay. However, the significance of these factors has not been studied. In the study described here, I examined these and other factors that could affect the glass-vial bioassay in an effort to produce a more standard bioassay for plant bugs. This standard bioassay was then used to determine insecticide resistance levels (in 3 groups of plant bugs) to 10 insecticides commonly used for plant bug control in cotton.

### Materials and Methods

Adult tarnished plant bugs were obtained from a colony that has been in continuous culture at the Southern Insect Management Laboratory, USDA-ARS, Stoneville, MS, since October 1988. This colony was reared on green beans and broccoli, *Brassica oleracea* L. variety *botrytis* L., by using methods described by Snodgrass and McWilliams (1992), and adults collected from weeds at Stoneville each spring were added to the colony to maintain vigor. Adults used in each test were aged for 3–7 d and were not tested separately by sex unless otherwise stated. Nymphs were not tested because of the large differences in size in the 5 nymphal stages. Glass scintillation vials (20 ml) were used in all tests.

Insecticide was applied to each vial by pipetting 0.5 ml of an insecticide 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, candidate insecticides were applied to the vials on the same day the test was performed. Technical grade insecticides were used. Acephate, cypermethrin, dicrotophos, fenvalerate, malathion, and methyl parathion were purchased from Chem Service, West Chester, PA; all were  $\geq 98\%$  pure. Dimethoate (96–98% pure) was obtained from Cheminova Agro A/S, Lemvig, Denmark. Bifenthrin, endosulfan, and permethrin (91.9, 95.0, and 94.7% pure, respectively) were obtained from FMC, Princeton, NJ.

Adult plant bugs also were collected with a sweep net from the commonly occurring wild host plants *Erigeron annuus* (L.) Persoon and *E. philadelphicus* L. in May and June at Stoneville, MS and near Crossett, AR. Plant bugs collected at Stoneville were of the  $F_1$  and  $F_2$  generations that would move into cotton fields near Stoneville in May and June. However, bugs collected from Crossett, where cotton is not grown, were from a population susceptible to most insecticides used in cotton. Resistance levels in bugs from wild host plants from both locations (and in bugs from the laboratory colony) were estimated for the 10 in-

secticides (listed previously) by using the glass-vial bioassay developed in this study. Plant bugs collected from *E. annuus* and *E. philadelphicus* were held in the laboratory and fed green beans for 1–2 d before tests. This allowed those bugs injured when collected from wild hosts to die before being used in the tests.

Green beans used as food in the vials were purchased at local food stores. Before they were used in a test, green beans were soaked for 30 min in a solution made by adding 75 ml of 3% sodium hypochlorite to 11 liters of water. The beans were then washed in detergent, after which they were thoroughly rinsed in clean water. This treatment surface-sterilized the beans and removed or oxidized any pesticide residue that may have been present. Mortality caused by contaminated green beans was never detected in any test. The green beans were cut transversely into pieces  $\approx 3$  mm thick, and 1 piece was used in each vial. Before use in the vials, bean pieces were dried on tissue paper. After plant bugs were placed in a vial, a cotton ball was placed in the vial opening to confine the bugs. During a test the vials were held in an upright position at laboratory conditions of 24–26°C, and humidity was not controlled. A plant bug was considered dead when it was unable to right itself or walk, or when it did not move when prodded. Glass-vials used in the tests were cleaned and reused. Dirty vials were soaked for at least 24 h in a detergent solution, then each was scrubbed with a brush. Vials were then rinsed 4 times in tap water; these rinses were followed by an acetone rinse to remove any soap or insecticide residue, and finally a distilled water rinse. Vials were then baked at 220°C for 2 h in a laboratory oven (Imperial IV, Lab-Line Instruments, Melrose Park, IL) to destroy any remaining residues.

**Age and Sex Test.** To study the possible effects of age and sex on mortality of plant bugs in the bioassay, new adults ( $\approx 250$  of each sex) were collected from the colony over a 2-d period, their sex was determined, and they were held by sex on green beans. The separate sexes were held until 2–3, 9–10, and 16–17 d of age, then tested by placing them into vials that had been treated with 5  $\mu$ g of permethrin per vial (a dose determined in preliminary tests to kill 50–60% of the bugs). Two adults of the same sex were used per vial, and each vial had a piece of green bean for food. Experimental design was a randomized complete block with 3 replications. Each replication included 20 vials (10 with males and 10 with females). Data were analyzed using analysis of variance (ANOVA) and means were separated by least significant difference (LSD) at  $P < 0.05$  (SAS Institute 1989) where appropriate.

**Food and Number of Adults per Vial.** The effect of food (green bean) on mortality in the bioassay was studied in 3 tests. In the 1st test, adults from the laboratory colony were placed in vials that had been treated with 0.5 ml of acetone, and a

piece of green bean was added. One adult was placed into each of 100 vials; 50 vials were used in each of the treatments with 2 and 3 adults per vial. Experimental design was completely randomized. In the 2nd test, the vials were treated with 0.5 ml of acetone, but no food was placed in the vials. Experimental design was a randomized complete block with 5 vials of each treatment in each replication and 10 replications. Mortality was determined in both tests after adults were held in the vials for 24 h. The 3rd test was done to determine mortality in bugs held with food in vials treated with insecticide and 1, 2, or 3 adults per vial. Vials were treated with permethrin (8.0  $\mu\text{g}$  per vial), a piece of green bean was added, and 1, 2, or 3 laboratory colony adults were added to each vial. For each treatment (1, 2, or 3 adults per vial), 4 replicates of 10 vials were used. Experimental design was a randomized complete block and mortality was determined 24 h after the adults were placed in the vials. This test was performed daily over 3-d. Data from all 3 tests were analyzed with the generalized linear model (PROC GLM; SAS Institute 1989); treatment means were compared by least significant difference (LSD) ( $P < 0.05$ ; SAS Institute 1989) where appropriate.

**Mortality and Vial Exposure Time.** To determine if mortality and resulting  $\text{LC}_{50}$  values changed with increasing periods of exposure in the vials, vials were treated at rates of 0, 2.5, 5.0, 7.5, 10.0, 15.0, 25.0, and 50.0  $\mu\text{g}$  of permethrin. Three replications were used; in each replication, 10 vials were treated at each of the 8 rates of permethrin. Two laboratory colony adults were placed in each vial. Half of the vials (5) contained food (green bean), whereas no food was used in the remaining half. Mortality was determined after the bugs were exposed for 2, 4, 6, 8, 10, 12, 14, and 24 h in the vials. Mortality from each exposure period was used to estimate  $\text{LC}_{50}$  values for either fed or unfed adults assuming the probit model (Proc Probit; SAS Institute 1989). Both sets of  $\text{LC}_{50}$  values (from fed or unfed adults) were modeled as a function of time by loglinear regression (SAS Institute 1989).

**Adult Behavior in Vials.** Adults were studied to determine how their behavior was affected by exposure to insecticide in the vials. Effects of using 1 or 2 adults per vial were also studied. The test was done over 4 consecutive 2-h periods on each test day. In each of these 2-h periods, 5 time intervals of 15 min were used to observe bugs in each of 4 treatments. In one treatment, 1 adult was placed in a vial treated with 2.5  $\mu\text{g}$  of permethrin. In the 2nd treatment, 1 adult was placed in a vial treated only with acetone. In 2 other treatments, 2 adults were placed in each vial either treated with permethrin or acetone only. Vials in all treatments had a piece of green bean, and adults of the same age from the laboratory colony were used. Two observers watched 2 vials each, and the behavior was recorded as the amount of time spent

**Table 1. Mortality of adult tarnished plant bugs by sex and by age in glass vials treated with 5  $\mu\text{g}$  permethrin per vial**

| Age, d | % mortality <sup>a</sup> |        |       |
|--------|--------------------------|--------|-------|
|        | Male                     | Female | Avg   |
| 2-3    | 60.0a                    | 61.7a  | 60.9a |
| 9-10   | 56.7a                    | 53.3a  | 55.0a |
| 16-17  | 86.7b                    | 81.7b  | 84.2b |
| Avg.   | 67.8a                    | 65.6a  | —     |

Means by sex or by average in rows and columns followed by the same letter are not significantly different ( $P = 0.05$ ; least significant difference test [SAS Institute 1989]). LSD by sex for rows and columns is 10%; LSD by row and column average is 5.8 and 7.1%, respectively.

<sup>a</sup> Determined after 24 h of exposure in the vials.

by each adult on the inner glass surface of the vial. In vials with 2 adults, a dot of silver paint was placed on the pronotum of one of the adults so that behavior of each bug could be recorded separately. The test design was a randomized complete block with 3 replications. Each replication was the observations made during the 8-h period in a test day. Data were analyzed by ANOVA (SAS Institute 1989), where the 2 adults per vial treatments were treated as subsamples. Treatment means were separated using LSD ( $P < 0.05$ ). Appropriate LSD values were calculated to account for unbalanced (1 or 2 adults per vial) data.

**Use of the Bioassay.** The glass-vial bioassay developed from results of the previously described tests was used to determine resistance levels to 10 insecticides commonly used in cotton in the 3 groups of plant bugs described previously. At least 6 doses of insecticide (as many as 12) were tested with each insecticide, each test was replicated 3 or 4 times, and each replication had 5 vials each containing 2 or 3 adults. Control vials were treated only with acetone, and control mortality was rare and never  $>3\%$ . Data were corrected for control mortality using Abbott's (1925) formula before analysis. Data from these glass-vial bioassays were analyzed assuming the probit model (Proc Probit; SAS Institute 1989). Among the 3 groups of plant bugs studied, differences in  $\text{LC}_{50}$  values for each insecticide were considered significant if the 95% CL of the resistance ratio at  $\text{LC}_{50}$  did not include 1.0 (Robertson and Preisler 1992).

## Results and Discussion

**Age and Sex Test.** Mortality in the vials treated with 5  $\mu\text{g}$  of permethrin was not significantly affected ( $F = 0.39$ ;  $\text{df} = 1, 4$ ;  $P > F = 0.566$ ) by the sex of the laboratory-reared adults (Table 1). However, age of the adults did significantly influence the results. Adults tested at 16-17 d old had significantly higher mortality ( $F = 73.50$ ;  $\text{df} = 2, 8$ ;  $P > F = 0.0001$ ) than those tested when 2-3 or 9-10 d old. These results indicated that, if laboratory-reared adults are used in a glass-vial bioassay,

say, they should be used before they were 10 d old. The age of field-collected plant bugs cannot be determined unless nymphs are collected and reared in the laboratory. Sex of the plant bugs was not a significant factor in this test. Leigh and Jackson (1968) found that mortality by topical application at a given insecticide dose was greater for males than for females of *L. hesperus* because of the higher average weight of the females. Female *L. lineolaris* also are larger than males; however, significant differences in mortality in males or females were not detected in my test.

**Food and Number of Adults per Vial.** Survival of adult plant bugs in the acetone-treated vials was high over a 24-h period when 1, 2, or 3 adults were placed in vials with a food source. Initial analysis of the data indicated that the vial component of error compared with the insects within vial error was not significant ( $F < 1$ ). Therefore, each insect was treated as a replication to simplify the analysis. Mean survival ( $\pm$ SEM) for the treatments with 1, 2, and 3 adults per vial was 98.0 (0.02), 97.0 (0.02), and 97.3% (0.01), respectively, and I found no significant differences caused by the treatments ( $F = 0.10$ ;  $df = 2, 347$ ;  $P > F = 0.90$ ). In the 2nd test, survival in the acetone-treated vials with no food was 76.0 (0.05), 83.0 (0.05), and 84.7% (0.05) for the treatments with 1, 2, and 3 adults per vial, respectively. No significant differences ( $F = 0.96$ ;  $df = 2, 9$ ;  $P > F = 0.403$ ) were found among the treatments. Mortalities of  $\leq 3\%$  in the 1st test showed that adult survival in the glass-vials with food over a 24-h period would be high in control treatments. However, mortalities of 24.0, 17.0, and 15.3% for the treatments with 1, 2, and 3 adults per vial without food in the 2nd test are too high to be used as control treatments. The higher survival in the treatments with 2- and 3-adult per vial indicate that some scavenging of dead adults may have occurred.

Numbers of adults used in the vials containing food and treated with 8  $\mu$ g of permethrin affected mortality. This test was done 3 times to check its repeatability; preliminary analysis indicated that the treatment-by-test interaction was not significant ( $F < 1$ ). Therefore, data were analyzed as a randomized complete block with 12 replications. Mean mortality was 71.7, 66.3, and 48.3% in the treatments with 3, 2, and 1 adults per vial, respectively. Mortality of 48.3% in the treatment with 1 adult per vial was significantly lower ( $F = 24.54$ ;  $df = 2, 22$ ;  $P > F = 0.0001$ ) than mean mortality in the treatments with 2 and 3 adults per vial, which were not significantly different. The higher mortalities in the treatments with 2 or 3 adults per vial showed that the presence of  $>1$  adult per vial significantly affected mortality in the vials. Possible causes for this increase in mortality are presented in the following results of the test on adult behavior in the vials.

**Adult Behavior in Vials.** Mean times spent on the treated (2.5  $\mu$ g permethrin) or untreated (ac-

**Table 2.** Average time for adult tarnished plant bugs on the inner glass surface of treated glass vials when 1 or 2 adults were placed in the vials with a food source

| Treatment no. | Glass vial treatment |                          | Time period observed <sup>b</sup> | Mean time on glass surface, min | LSD <sup>c</sup> |
|---------------|----------------------|--------------------------|-----------------------------------|---------------------------------|------------------|
|               | <i>n</i>             | Insecticide <sup>a</sup> |                                   |                                 |                  |
| 1             | 1                    | A                        | 1                                 | 1.65a                           | 7.36             |
| 2             | 1                    | P                        | 1                                 | 3.44b                           | 5.73             |
| 3             | 2                    | A                        | 1                                 | 4.64c                           | 3.38             |
| 4             | 2                    | P                        | 1                                 | 9.81abc                         | —                |
| 1             | 1                    | A                        | 2                                 | 0.00a                           | 5.10             |
| 2             | 1                    | P                        | 2                                 | 1.44b                           | 4.17             |
| 3             | 2                    | A                        | 2                                 | 2.00c                           | 2.96             |
| 4             | 2                    | P                        | 2                                 | 9.11abc                         | —                |
| 1             | 1                    | A                        | 3                                 | 1.67                            | NS               |
| 2             | 1                    | P                        | 3                                 | 3.20                            | —                |
| 3             | 2                    | A                        | 3                                 | 2.10                            | —                |
| 4             | 2                    | P                        | 3                                 | 6.20                            | —                |
| 1             | 1                    | A                        | 4                                 | 2.57                            | NS               |
| 2             | 1                    | P                        | 4                                 | 0.33                            | —                |
| 3             | 2                    | A                        | 4                                 | 2.18                            | —                |
| 4             | 2                    | P                        | 4                                 | 5.33                            | —                |

Means within each time period followed by a, b, or c are significantly different ( $P < 0.05$ ; LSD test [SAS Institute 1989]; NS, no significant difference).

<sup>a</sup> A, 0.5 ml of acetone only; P, 2.5  $\mu$ g of permethrin in 0.5 ml of acetone per vial.

<sup>b</sup> Adults were observed over an 8-h period divided into 4 periods of 2 h each. The consecutive 2-h periods were numbered 1, 2, 3, and 4; within each 2-h period, observations were made in 5 periods of 15 minutes each.

<sup>c</sup> Three LSD values are listed for each time period. The 1st value is for comparison of treatments 1 and 2, the 2nd for comparison of treatments 1 and 2 with treatments 3 and 4, the 3rd for comparison of treatments 3 and 4.

etone only) inner glass surfaces of the vials by adults in the 4 treatments were significantly different in the 1st ( $F = 5.7$ ;  $df = 3, 6$ ;  $P > F = 0.04$ ) and 2nd ( $F = 14.7$ ;  $df = 3, 6$ ;  $P > F = 0.004$ ) time periods of the test (Table 2). In both treated and untreated vials with only 1 adult, plant bugs spent significantly shorter mean times on the treated or untreated surfaces than did the 2 adults in treated vials during both the 1st and 2nd periods. Two adults in the untreated vials also spent significantly shorter mean times on the untreated glass than the 2 adults in the treated vials in these 2 periods. Mean times spent on the treated and untreated surfaces of the vials in all 4 treatments were not significantly different in the 3rd ( $F = 1.1$ ;  $df = 3, 6$ ;  $P > F = 0.41$ ) or 4th ( $F = 0.71$ ;  $df = 3, 6$ ;  $P > F = 0.58$ ) periods. The presence of a 2nd adult influenced the behavior of both adults. The 2 adults frequently ran into each other on the bean, which caused movement of 1 or both adults from the bean to the surface of the vial. In the untreated vials with 2 adults, mean time spent by adults on the glass surface was shorter in every period compared with the 2 adults in the permethrin-treated vials. Being poisoned with permethrin apparently caused additional movement of adults from the bean and resulted in an observed greater amount of time spent on the treated glass surface. This effect was also observed in the per-

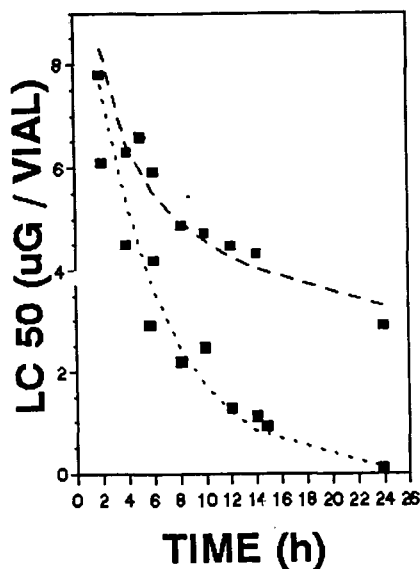


Fig. 1. Decrease in  $LC_{50}$  values with increasing time for adult tarnished plant bugs exposed to 8 rates of permethrin in glass-vials. Food (green bean) was present [upper line,  $\log X = 2.37$  ( $\log Y$ ),  $R^2 = 0.95$ ] or absent [lower line,  $\log X = 2.39 - 0.184 (Y)$ ,  $R^2 = 0.97$ ] from each vial. ■, observed  $LC_{50}$  values.

methrin-treated vials with 1 adult (that is single adults in 3 of the 4 periods in permethrin-treated vials spent a longer mean amount of time on the glass surface than did single adults in the untreated vials). Mean times for adults on the glass surface in the permethrin-treated vials decreased in time periods 3 and 4, probably as a result of accumulation of poison in the adults which affected their muscular coordination and ability to move. Results from this and the previous test indicate that at least 2 adults should be used per vial to ensure their exposure to the treated surface of the vial.

**Mortality and Vial Exposure Time.** Data on how  $LC_{50}$  values changed in time using vials with food were fit equally well with linear regression when the logarithms of the  $LC_{50}$  values were regressed over the times, or when they were regressed over the logarithms of the times (both  $R^2$  values were 0.95). Regression of the logarithms of the  $LC_{50}$  values over the logarithms of the times is shown in Fig. 1. Data comparing  $LC_{50}$  values in time, when the vials had no food, were best fit by linear regression of the logarithms of the  $LC_{50}$  values over time ( $R^2 = 0.97$ ). The  $F$  test for homogeneity of slopes (Steel and Torrie 1980) was done with slopes from the regressions of the logarithms of the  $LC_{50}$  values over time to determine if the trends (the decrease in the  $LC_{50}$  values over time) of the 2 lines were different. The slopes were significantly different ( $F = 102$ ;  $df = 1, 12$ ;  $P > F = 0.0001$ ) indicating that  $LC_{50}$  values over time decreased at significantly different rates. The final  $LC_{50}$  values for plant bugs exposed in the vials for

24 h were 2.91 and 0.10  $\mu\text{g}$  per vial for vials with and without food, respectively. Presence of food made a 29-fold difference in the  $LC_{50}$  values at 24 h. These results showed that both the presence of food and length of time adults were exposed in the vials significantly affected mortality.

Based on all tests I adapted the following glass-vial bioassay procedure. Adult plant bugs were tested when they were  $<10$  d old (if the age was known) and bugs were not tested separately by sex. Food (green bean) was used in each vial with 2 or 3 adults per vial, and mortality was determined after a 24-h exposure period. Bioassays with tarnished plant bugs that do not include food in the vials or use shorter exposure periods are also possible. In my study, a 24-h exposure period was used, because it allowed time for treating the vials and placing food and adults into the vials. Ideally, glass-vials treated with different classes of insecticides at desired concentrations could be stored until needed. This has been done with glass-vials used in monitoring resistance in *H. virescens* (Kanga et al. 1993).

**Use of the Bioassay.** Tarnished plant bugs from the 3 locations (laboratory colony, or collected from *Erigeron* spp. near Stoneville, MS, or Crossett, AR) were all susceptible to the pyrethroid insecticides bifenthrin, cypermethrin, and permethrin (Table 3). However, fenvalerate had  $LC_{50}$ s as much as 50 times higher (37.05 versus 0.67  $\mu\text{g}$  per vial) than the  $LC_{50}$ s found with the other pyrethroids. No significant differences in  $LC_{50}$ s were found among plant bugs from 2 of the locations for bifenthrin, whereas plant bugs collected near Crossett had a significantly higher  $LC_{50}$  than those collected at Stoneville for permethrin. For cypermethrin and fenvalerate, plant bugs collected at Stoneville had significantly higher  $LC_{50}$ s as compared with plant bugs from the laboratory colony and those collected near Crossett.

Among the organophosphate insecticides tested, methyl parathion was the most toxic with an  $LC_{50}$  as low as 0.26  $\mu\text{g}$  per vial for plant bugs from Crossett (Table 3). I found no significant differences in susceptibility to methyl parathion in the plant bugs from any of the 3 locations. Plant bugs in the laboratory colony were generally more tolerant to the organophosphates tested than plant bugs from the other 2 locations. The  $LC_{50}$ s for the laboratory colony bugs were significantly higher than the  $LC_{50}$ s for plant bugs collected at Stoneville. Plant bugs collected from Crossett had  $LC_{50}$ s significantly higher than those found for bugs collected at Stoneville for acephate and dimethoate. These results, along with those for permethrin from bugs collected at Crossett, were reverse of what I expected. The reason(s) for this higher level of tolerance in bugs that were expected to be susceptible is unknown.

Data obtained from bioassays of the 3 groups of plant bugs tested with the 10 insecticides is the largest set of data currently available on insecticide

Table 3. Mortality of adult tarnished plant bugs exposed to 10 insecticides in a glass vial bioassay

| Collection <sup>a</sup><br>location | Insecticide              | n   | Slope $\pm$ SE <sup>b</sup> | LC <sub>50</sub> | 95% CL      | $\chi^2$ | Comparison<br>location | RR <sub>50</sub> <sup>c</sup> | 95% CL                            |
|-------------------------------------|--------------------------|-----|-----------------------------|------------------|-------------|----------|------------------------|-------------------------------|-----------------------------------|
| C                                   | Bifenthrin               | 480 | 0.78 $\pm$ 0.08             | 1.15             | 0.89–1.42   | 5.34     | —                      | —                             | —                                 |
| S                                   | Bifenthrin               | 480 | 0.57 $\pm$ 0.06             | 1.10             | 0.77–1.46   | 6.56     | —                      | —                             | —                                 |
| C                                   | Cypermethrin             | 210 | 1.21 $\pm$ 0.15             | 1.41             | 1.13–1.72   | 2.58     | C                      | 1.05                          | 0.70–1.55                         |
| SLC                                 | Cypermethrin             | 270 | 0.76 $\pm$ 0.12             | 0.67             | 0.40–0.94   | 0.61     | SLC                    | 2.10                          | 1.33–3.35                         |
| S                                   | Cypermethrin             | 240 | 1.38 $\pm$ 0.20             | 1.97             | 1.75–2.59   | 2.28     | —                      | —                             | —                                 |
| SLC                                 | Fenvalerate              | 210 | 1.17 $\pm$ 0.15             | 24.01            | 19.66–29.23 | 6.45     | SLC, C                 | 2.94, 1.40                    | 1.86–4.64, 1.04–1.86              |
| S                                   | Fenvalerate              | 390 | 1.06 $\pm$ 0.10             | 22.32            | 18.99–26.18 | 9.33     | SLC                    | 1.08                          | 0.69–1.90                         |
| SLC                                 | Permethrin               | 480 | 1.14 $\pm$ 0.11             | 37.05            | 31.84–43.08 | 12.34    | —                      | —                             | —                                 |
| S                                   | Permethrin               | 240 | 1.01 $\pm$ 0.08             | 3.39             | 2.87–3.99   | 7.09     | SLC, C                 | 1.66, 1.54                    | 1.33–2.07, 1.21–1.98              |
| C                                   | Permethrin               | 405 | 1.19 $\pm$ 0.21             | 2.91             | 1.94–3.72   | 3.44     | S, SLC                 | 1.32, 1.17                    | 1.05–1.66, 0.83–1.65              |
| SLC                                 | Endosulfan               | 480 | 1.21 $\pm$ 0.10             | 2.57             | 2.19–3.01   | 5.93     | S                      | 1.13                          | 0.81–1.59                         |
| C                                   | Endosulfan               | 480 | 1.08 $\pm$ 0.18             | 11.67            | 7.61–17.51  | 11.18    | —                      | —                             | —                                 |
| SLC                                 | Accephate                | 420 | 0.70 $\pm$ 0.09             | 9.50             | 6.07–14.50  | 13.0     | SLC                    | 1.23                          | 0.78–1.93                         |
| S                                   | Accephate                | 240 | 3.16 $\pm$ 0.42             | 8.46             | 7.39–9.57   | 10.21    | —                      | —                             | —                                 |
| SLC                                 | Accephate                | 270 | 1.84 $\pm$ 0.24             | 12.60            | 11.06–14.51 | 5.39     | S                      | 1.40                          | 1.16–1.69                         |
| S                                   | Dicrotophos <sup>d</sup> | 270 | 1.39 $\pm$ 0.17             | 6.05             | 5.04–7.04   | 6.27     | S, C                   | 2.08, 1.49                    | 1.69–2.57, 1.27–1.74              |
| C                                   | Dicrotophos <sup>d</sup> | 240 | 0.93 $\pm$ 0.19             | 2.17             | 1.57–2.88   | 7.53     | —                      | —                             | —                                 |
| SLC                                 | Dimethoate               | 270 | 1.58 $\pm$ 0.19             | 1.33             | 1.15–1.53   | 7.16     | S                      | 1.63                          | —                                 |
| S                                   | Dimethoate               | 330 | 2.57 $\pm$ 0.26             | 1.17             | 1.01–1.33   | 2.45     | —                      | —                             | —                                 |
| C                                   | Dimethoate               | 210 | 2.06 $\pm$ 0.28             | 2.28             | 2.03–2.52   | 12.31    | S                      | 1.46                          | 1.15–1.87                         |
| SLC                                 | Malathion                | 210 | 1.59 $\pm$ 0.24             | 0.80             | 0.63–1.00   | 3.70     | S, C                   | 2.85, 1.95                    | 2.28–3.59, 1.64–2.32              |
| S                                   | Malathion                | 270 | 1.29 $\pm$ 0.17             | 4.13             | 3.29–5.02   | 4.37     | —                      | —                             | —                                 |
| C                                   | Malathion                | 210 | 1.11 $\pm$ 0.19             | 5.44             | 3.64–7.99   | 15.72    | S                      | 1.21                          | 0.90–1.63                         |
| SLC                                 | Methyl parathion         | 270 | 1.31 $\pm$ 0.17             | 3.41             | 2.70–4.17   | 1.77     | S, C                   | 1.60, 1.32                    | 1.11–2.29, 0.92–1.89              |
| S                                   | Methyl parathion         | 240 | 0.90 $\pm$ 0.11             | 0.26             | 0.21–0.34   | 4.81     | —                      | —                             | —                                 |
| SLC                                 | Methyl parathion         | 300 | 1.73 $\pm$ 0.23             | 0.33             | 0.29–0.38   | 1.64     | —                      | —                             | —                                 |
| S                                   | Methyl parathion         | 300 | 0.90 $\pm$ 0.10             | 0.27             | 0.21–0.34   | 4.09     | S, D<br>C              | 1.22, 1.38<br>1.13            | 0.93–1.61, 0.88–2.23<br>0.69–1.90 |

<sup>a</sup> Adults tested were from a laboratory colony (SLC) maintained at Stoneville, MS, or were collected from *Erigeron* spp. growing near cotton fields at Stoneville (S), and in weedy areas near Crossett, AR (C) (where cotton is not grown commercially).

<sup>b</sup> Insecticide concentrations are  $\mu\text{g}$  per vial; survival was scored at 24 h.

<sup>c</sup> Resistance ratio: LC<sub>50</sub> of collection location divided by LC<sub>50</sub> of comparison location.

<sup>d</sup> The data for dicrotophos were best fit using probit analysis on original (nonlogarithmic) mortalities. Therefore, CI of the difference in the LC<sub>50</sub> value (0.84) was calculated as 0.37–1.31. The LC<sub>50</sub> values were significantly different at  $P = 0.05$  because the confidence interval did not include 0.

tolerance in the tarnished plant bug. The highest resistance ratio found for all insecticides tested was 2.94 (Table 3, cypermethrin); most were between 1 and 2. These data can be compared with results from bioassays for plant bugs collected from cotton fields in which control with insecticides was inadequate. This was done in 1993 (Snodgrass 1994), when a tarnished plant bug population was found in cotton near Schlater, MS, with resistance levels >30 times higher than those found in plant bugs collected from *Erigeron* spp. at Stoneville for permethrin and bifenthrin.

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