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EFFECT OF ULV MALATHION USE IN BOLL WEEVIL ERADICATION ON RESISTANCE IN THE TARNISHED PLANT BUG

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Abstract

Tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), from Regions 1 (north Delta), 2 (south Delta), and 3 (hills) of the boll weevil, *Anthonomous grandis* Boheman, eradication program in Mississippi were collected from wild hosts and tested for malathion resistance in the fall of 1999 and during the spring and fall of 2000 and 2001. In Region 1, plant bugs were also tested in late-July of 1999, just prior to the start of the multiple applications of ULV malathion used for reproduction-diapause control of boll weevils in August. Regions 1, 2, and 3 began boll weevil eradication in 1999, 1998, and 1997, respectively. A glass-vial bioassay was used to determine resistance in plant bugs to malathion, and LC_{50} values obtained were compared to the LC_{50} value obtained for susceptible plant bugs. Comparison of the LC_{50} value obtained for plant bugs at a location in the spring also was made to the LC_{50} value obtained in the fall at the same location. Applications of malathion made for reproduction-diapause boll weevil control increased malathion resistance in plant bugs from July of 1999 to October of 1999 by 4.9-, 6.5-, and 20.8-fold at the three locations where plant bugs were tested in Region 1. Results from testing plant bugs from all three eradication regions were similar. Malathion resistance usually increased significantly from spring to fall, then declined significantly from fall to spring. Despite greatly reduced use of malathion in all three eradication regions for boll weevils in 2001 (due in part to a cold winter in 2000-2001), resistance to malathion in plant bugs still increased significantly from spring to fall at all test locations in Regions 1 and 2 (the Delta). The increase in malathion resistance from spring to fall in Regions 1 and 2 in 2001 is thought to be the result of extensive use of organophosphate insecticides for plant bugs in the Delta in 2001. Plant bugs were a minor problem in Region 3 (the hills) in 2001, and malathion resistance did not increase significantly in plant bug populations from spring to fall at three of four test locations in this year. Overall test results showed that the use of malathion in boll weevil eradication can rapidly produce several fold increases in resistance to malathion in plant bug populations. However, the expression of this resistance was usually rapidly lost. The reason(s) for the loss of the expression of the resistance is unknown.

Introduction

Boll weevil, *Anthonomous grandis* Boheman, eradication is currently being conducted in all cotton growing areas of Mississippi. The state was split into four regions for eradication, and eradication began in each region in August of its first year with a reproduction-diapause control program. The reproduction-diapause program consists of multiple applications (typically ten) of ULV malathion. From August through early-September, applications are made at short intervals (4-5 d) to control reproductive females. These are followed by applications at longer (7-14 d) intervals to control diapausing boll weevils. Region 4 of the eradication program in Mississippi is a one to three county wide strip extending from north to south from the Tennessee line to the Gulf Coast. It began the current eradication program in August of 1997. Region 3 is all of the hill country of Mississippi west of Region 4, and it also began eradication in August of 1997. Region 2 is the south portion of the Yazoo-Mississippi River Delta and eradication began in this region in August of 1998. Region 1 is the Delta north of Region 2, and eradication began in Region 1 in August of 1999.

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is an economically important pest of cotton, *Gossypium hirsutum* L., in the southeastern United States. Control of tarnished plant bugs in cotton is achieved almost exclusively with insecticides. Tarnished plant bug populations in all areas of the Delta have developed resistance to pyrethroid insecticides, and pyrethroid resistant populations also have increased tolerance to several organophosphate, carbamate, and cyclodiene insecticides (Snodgrass and Elzen 1995, Pankey et al. 1996, Snodgrass 1996a, Hollingsworth et al. 1997, Snodgrass and Scott 2000). The widespread use of malathion for boll weevil eradication could increase tolerance in plant bugs to this insecticide as well as to other organophosphorus insecticides. Organophosphate insecticides (including malathion) are commonly used for plant bug control in Mississippi, although the efficacy of the organophosphate insecticides for plant bugs in cotton in Mississippi has declined (Howell et al. 1998). Acephate is among the most commonly used organophosporous insecticides in cotton in Mississippi, and is very important to control of plant bugs since populations of pyrethroid resistant plant bugs are still susceptible to acephate but have increased tolerance to several other organophosphate insecticides (Snodgrass and Scott 2000).

An increase in tolerance to acephate and other organophosphorus insecticides in an area resulting from widespread use of malathion in boll weevil eradication would make plant bug control more difficult in that area.

ULV malathion applications in cotton for boll weevil control have been estimated to reduce tarnished plant bug populations by as much as 50% (Allen and Kharboutli 2000), however, 2-3 applications may be needed. The use of multiple applications of ULV malathion for control of boll weevils places a selection pressure for resistance on tarnished plant bug populations. Reproduction-diapause applications begin in August at which time a high percentage of the total plant bug population is normally in cotton in the Mississippi River Delta (Snodgrass et al. 1984, Luttrell et al. 1998). In addition, the ULV malathion concentration on cotton leaves can increase with sequential aerial applications (Wolfenbarger et al. 2000) and produce a constant selection pressure. The present study was conducted to study the effect of ULV malathion applications used in boll weevil eradication on malathion resistance in tarnished plant bugs.

Materials and Methods

Tarnished plant bugs were collected for resistance testing from wild host plants using a sweep net. Collection sites were selected at which wild hosts and plant bugs would be present in the spring and in the fall. These sites were usually undisturbed areas near drainage ditches. The same sites were used in all three years. Bugs were aspirated from the sweep net after collection in the field and placed into paper ice cream cartons (0.95 liter) with green beans, *Phaseolus vulgaris* L. The beans were washed in detergent and soaked in a 3% sodium hypochlorite solution as described in Snodgrass (1996b) to remove or oxidize any insecticide residue on them. The bugs were held in these containers under laboratory conditions of 24-26^o C and humidity was not controlled for a 24-h period before being tested to allow any injured bugs to die. A glass-vial bioassay was used to determine the amount of insecticide resistance present in the plant bug populations tested. A description of this bioassay is found in Snodgrass (1996b). In this bioassay, adult plant bugs were placed into 20-ml glass scintillation vials (2 per vial) that had been treated with malathion. The malathion was applied by pipetting 0.5 ml of insecticide diluted in acetone into each vial. Each vial was rolled on its side until an even layer of malathion dried on its inner surface. Vials in control treatments received only 0.5 ml of acetone, and in all tests the malathion was applied to the vials on the same day the test was performed. A small piece of green bean (washed and sterilized as described previously) approximately 3 mm thick (cut transversely) was added to each vial as food for the adults, and a cotton ball was used to seal each vial.

For each test, vials were held during a test in an upright position at laboratory conditions of 24-26^{o}C and humidity was not controlled. Mortality was determined after 24 h, and adults were considered dead if they were unable to right themselves or walk, or there was no movement when they were prodded. All bioassays had 3-4 replications of 6-13 different concentrations of malathion, and in each replication were 5 vials with 2 adults per vial. Technical grade malathion (98% pure) was used in the tests and was purchased from Chem Service, West Chester, PA. Data from glass-vial bioassays were analyzed with the PROC Probit option of SAS (SAS Institute 1989). Differences in LC_{50} values were considered significant if the 95% CL of the resistance ratio at the LC_{50} level did not include 1.0 (Robertson and Priesler 1992). The LC_{50} obtained with malathion at each location was compared to the LC_{50} for malathion obtained from testing susceptible bugs collected near Crossett, AR in November of 1999. Other comparisons were made by comparing the LC_{50} for malathion obtained in the fall at a location to that obtained in the spring of the same or following year at the same location.

Collection and testing of plant bugs in the spring of each year were completed from late-April through May. Plant bug collection and testing in the fall were done from mid-October through mid-November. However, adults were collected and tested in 1999 in the last week of July at the three locations used in Region 1. This testing was done prior to the initiation of ULV malathion applications in August for reproduction-diapause boll weevil control in Region 1. The three collection locations used in Region 1 were all in Washington County, MS and were located near Stoneville, Avon, and Winterville. The collection locations in Region 2 were near Delta City, Louise, Onward and Yazoo City in Sharkey, Humphreys, Sharkey, and Yazoo Counties, respectively. In Region 3, the collection locations were near Grenada, Holcomb, Oxberry, and Winona in Grenada, Grenada, Tallahatchie, and Montgomery Counties, respectively.

Results

Prior to the start of boll weevil eradication in Region 1, tarnished plant bugs tested from Avon and Winterville in July 1999 had little resistance to malathion, and their LC_{50}'s were not significantly different than the LC_{50} found for susceptible bugs from Crossett (Table 1). Plant bugs tested from Stoneville in July 1999 had significantly higher resistance to malathion than the susceptible bugs from Crossett. Malathion resistance in plant bugs tested from the three test locations in Region 1 increased after boll weevil eradication began in August of 1999. All but one of the comparisons made after July 1999 found significantly higher resistance to malathion in plant bugs collected at the three locations in Region 1 as compared to the susceptible bugs from

Crossett. Plant bugs tested from Avon in May 2001 did not differ significantly in malathion resistance from the susceptible bugs from Crossett, and plant bugs tested in the spring of both years at all three locations had resistance ratios of 3.2 or less as compared to the susceptible bugs. The effect that the multiple applications of ULV malathion in August and September 1999 for reproduction-diapause boll weevil control had on malathion resistance in plant bugs at the three locations in Region 1 can also be seen in the comparisons made between July and October of 1999. In all three comparisons the LC_{50} values found with malathion were significantly higher in October as compared to July. The resistance ratios were 4.9, 6.5, and 20.8 for Stoneville, Avon, and Winterville, respectively. In the eight comparisons made at the three test locations between LC_{50} values for malathion found in the fall with those found in the spring, the values found in the fall were significantly higher in all but two comparisons with significant resistance ratios ranging from 2.4 to 9.0. The Stoneville October 2000 vs April 2000 and April 2001 comparisons were not significantly different.

The LC_{50} values obtained from testing plant bugs from the four locations in Region 2 (Table 2) were all significantly higher than the LC_{50} value found for susceptible bugs from Crossett, except for three comparisons. The LC_{50}'s for malathion for plant bugs tested from Yazoo City in May 2000 and 2001, and Onward in May 2001, were not significantly different than the LC_{50} for susceptible bugs. Resistance ratios from comparisons with susceptible plant bugs in the spring at all locations in both years were 3.7 or lower except for plant bugs tested from Louise in May of 2000 whose resistance ratio was 11.9. Plant bugs tested from Louise and Delta City in November 2000 had the highest resistance ratios (30.5 and 24.1, respectively) as compared to the susceptible bugs from Crossett found in the study. In all comparisons at all locations between LC_{50} values for malathion obtained in the fall to those found in the spring at the same locations, the values found in the fall were significantly higher. Thus, the same pattern found in Region 1 was found in Region 2. Malathion resistance in tarnished plant bug populations was significantly higher in the fall after the cotton growing season as compared to malathion resistance found in the spring.

Ten of the LC_{50} values for malathion obtained by testing tarnished plant bugs from the four locations in Region 3 (Table 3) were significantly higher than the LC_{50} value for susceptible bugs from Crossett. In the remaining six comparisons (Grenada in November 2000 and May 2001; Winona in May 2000; and Holcomb in May 2000, and May and October 2001) the plant bugs had LC_{50}'s for malathion which were not significantly different from the LC_{50} for the susceptible plant bugs. All of the resistance ratios from comparisons with susceptible plant bugs in both years at all four locations in the spring (except for a 4.0 resistance ratio for Grenada in May of 2000) were less than two. Malathion resistance was lower in the fall in the plant bugs tested in Region 3 than the malathion resistance found in those tested from Regions 1 and 2. Six of the twelve comparisons between LC_{50} values for malathion obtained in the fall to those found in the spring at the same locations were not significantly different. These six comparisons were Grenada November 2000 vs May 2001, and October 2001 vs May 2001; Oxberry November 2000 vs May 2000 and 2001, and October 2001 vs May 2001; and Holcomb October 2001 vs May 2001. This compares to a lack of significance in two of the eight comparisons between fall and spring in Region 1, and in none of the twelve comparisons between fall and spring in Region 2. The highest resistance ratio found for any comparison of the LC_{50} values found for the plant bugs tested from the four locations in Region 3 was only 4.8. The pattern found for the comparisons of the LC_{50} values found for the plant bugs in Region 3 was similar to that found in these comparisons in Regions 1 and 2. Malathion resistance was significantly higher in the fall than in the spring in five of the comparisons, was not significantly different in six comparisons, and in one comparison (Grenada May 2000 vs November 2000) resistance declined from spring to fall.

Discussion

One factor that affected the amount of malathion used in boll weevil eradication in Mississippi was winter mortality of overwintering boll weevils. Records from the weather station at Stoneville (Delta Branch Experiment Station, Stoneville, MS) showed that in December of 1996, 1997, and 1999 the soil temperature 5.1 cm deep at Stoneville was never below freezing. In 1998 the temperature dropped to -1.1^0 C for one day, while in 2000 it was 0^0 C or less for six consecutive days in December. The below freezing temperatures for a six-day period probably increased overwintering boll weevil mortality, and this combined with the cumulative control obtained from the previous control efforts in the eradication program, greatly reduced the number of boll weevils and malathion used for their control in 2001. An average of 1.3, 0.9, and 1.4 applications per acre were made in 2001 in Regions 1, 2, and 3, respectively (personal communication Farrel Boyd, Program Manager, Mississippi Boll weevil Eradication Program). This compares to previous average applications per acre of 6.8 in 2000 in Region 1; 7.9 and 3.0 in 1999 and 2000 in Region 2; and 13.3, 8.3, and 4.9 in 1998, 1999, and 2000 in Region 3. Despite the reduced amount of malathion used for boll weevil control in 2001, resistance ratios found for malathion (as compared to susceptible bugs) at the eight locations in Regions 1 and 2 in October 2001 were still high. These ratios ranged from 8.6 to 17.6, as compared to the resistance ratios found for plant bugs tested in October 2001 in Region 3 which ranged from 1.3 to 4.8, with three of them 2.1 or less. The higher amount of malathion resistance found in the plant bugs tested in Regions 1 and 2 (the Delta) was probably the result of plant bug pressure in cotton during the growing season of 2001. Plant bug numbers were high in cotton in the Delta of Mississippi in 2001 and organophosphate insecticides were commonly used for their control. This compares to low pressure from plant bugs in cotton grown in Region 3 (the hills) in 2001. This follows the usual pattern of plant bug infestation of cotton found in Mississippi. The average number of insecticide applications made for plant bug control in cotton in the Delta from 1996-1999 ranged from 1.2 to 3.1, as compared to a range of 0.1 to 0.7 in the hills (Williams 1997-2000).

The testing of the tarnished plant bugs from the three eradication regions found a similar pattern for malathion resistance. Resistance to malathion usually increased significantly from spring to fall then declined significantly from fall to spring. Plant bugs tested from Louise (Region 2) in May of 2000 had a resistance ratio as compared to susceptible plant bugs of 11.9, which increased to 30.5 in the fall. This was the only location at which the resistance ratio found in the spring was greater than 4.0, and in most comparisons to susceptible plant bugs (12 of 20) at all locations in the spring, the resistance ratio was 2.0 or less. The ability of the plant bug population at Louise to express a fairly high amount of malathion resistance in the spring is important. Malathion used for boll weevil control in cotton infested with these bugs would probably be less effective in controlling the plant bugs. Resistance to malathion would also increase more rapidly, and in fact the highest resistance ratio found in the study (30.5) was found in October of 2000 at Louise. In the spring of 2001, the resistance ratio found for plant bugs from Louise had declined to 3.2. These results demonstrated that while high levels of resistance to malathion in plant bugs were usually lost from the fall of one year to the spring of the following year, its expression can be retained in some populations from one year to the next. Producers and consultants in areas undergoing boll weevil eradication where plant bugs are also a problem should not assume that applications of malathion made for boll weevil control will also control plant bugs. Fields should be sampled for plant bugs to determine if additional control measures are needed.

The basis for the malathion resistance has not been studied in tarnished plant bugs in the Delta. Some populations of a closely related species of plant bug, *Lygus hesperus* Knight, found in the Western United States have developed high levels of resistance to the organophosphate trichlorfon. This resistance was caused by enhanced esterase detoxification (Zhu and Brindley 1990a) and reduced acetylcholinesterase sensitivity (Zhu and Brindley 1990b). Nymphs of the tarnished plant bug begin developing into adults that are in reproductive diapause in mid-August in the Delta of Mississippi, and by October 90% or more of the nymphs present produce adults in diapause (G. L. S. unpublished data). Diapause is broken in December at which times the adults mate and females can oviposit viable eggs. In the winters of 1998-1999 and 1999-2000, nymphs were present on wild hosts in the Delta in January, and F_1 adults were found by mid-March. F_1 adults were not produced in the colder winter of 2000-2001 until the first week of April. The exact time required for plant bugs to develop naturally on wild hosts under the fluctuating temperatures found in the spring is not known. Ridgway and Gyrisco (1960) found that at constant temperatures of 20 and 25° C about 46 and 27 days, respectively, were needed for development from egg deposition to new adult using green beans as food. Tarnished plant bugs that we tested in late-April through May of 2000 were probably a mixture of F_1 and F_2 adults. In 2001, F_1 adults were found about three weeks later than in the previous year, and we probably tested mainly F_1 adults in the late-April through May test period. Overwintered adults are mostly dead by May, and the plant bugs we collected and tested in late-April through May were probably one or two generations removed from a parent generation that often possessed a much higher level of malathion resistance. If the basis for the insecticide resistance is genetic, our results showed that its expression was usually mostly lost after only one or two matings that occurred when the F_1 and F_2 generations were produced. The basis for the resistance to malathion is currently being studied.

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Table 1. Mortality of adult tarnished plant bugs exposed to malathion in a glass-vial bioassay. The three collection locations are all in Washington County, MS which is in Region 1 (north Delta) of the boll weevil eradication program in Mississippi. Reproduction-diapause applications of ULV malathion began in Region 1 in August of 1999.

Collection location	**Date collected**	***n***	**Slope + SE**[a]	**LC_{50}**	**95% CL**	**Comparison**[b]	**RR_{50}** [c]	**95% CL**
Stoneville	July 1999	210	2.1 + 0.28	16.6	14.7-18.4	C;	3.4	2.7-4.2
Stoneville	Oct.1999	390	0.7 + 0.07	81.8	65.8-102.4	C; July 99; Apr. 00	6.8; 4.9; 6.7	12.7-22.1; 3.87- 6.3; 4.7-9.6
Stoneville	Apr. 2000	210	0.7 + 0.15	12.2	8.6-15.9	C;	2.5	1.8-3.5;
Stoneville	Oct. 2000	270	0.8 + 0.11	18.2	14.3-22.7	C; Apr. 00; Apr. 01	3.7; 1.5; 1.2	2.8-5.0; 1.0-2.2; 0.8-1.7
Stoneville	Apr. 2001	210	0.6 + 0.11	15.8	10.7-21.9	C;	3.2	2.2-4.7
Stoneville	Oct. 2001	270	0.5 + 0.09	65.5	46.3-98.4	C; Apr.01	13.4; 4.2	9.0-20.0; 2.5-6.8
Avon	July 1999	210	1.1 + 0.15	3.7	3.0-4.5	C;	0.8	0.6-1.0
Avon	Oct. 1999	360	0.5 + 0.07	24.2	16.9-32.4	C; July 99	5.0; 6.5	3.5-7.1; 4.5-9.6
Avon	Oct. 2000	180	0.7 + 0.12	11.5	7.5-16.0	C; May 01	2.4; 2.4	1.6-3.5; 1.5-3.9
Avon	May 2001	240	0.8 + 0.09	4.7	3.5-6.2	C;	1.0	0.7-1.4
Avon	Oct. 2001	240	0.4 + 0.08	43.5	28.2-70.5	C; May 01	8.7; 9.0	5.5-13.9; 5.4-15.2
Winterville	July 1999	210	1.0 + 0.14	3.5	2.7-4.3	C;	0.7	0.5-1.0
Winterville	Oct. 1999	390	0.7 + 0.09	72.2	52.4-99.3	C; July 99	14.8; 20.8	10.7-20.4; 14.5-29.7
Winterville	Oct. 2000	270	0.3 + 0.07	36.6	17.3-61.6	C; May 01	7.5; 3.9	4.1-13.6; 2.0-7.4
Winterville	May 2001	210	0.9 + 0.14	9.5	6.6-12.2	C;	1.9	1.4-2.7
Winterville	Oct. 2001	240	0.6 + 0.09	42.1	30.6-60.8	C; May 01	8.6; 4.5	5.9-12.5; 2.9-7.0
Crossett	Nov. 1999	210	1.3 + 0.19	4.9	4.1-5.7			

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

[b] All LC_{50} values obtained at the three locations were compared to the LC_{50} value obtained for susceptible bugs collected and tested from Crossett, AR (C) in November of 1999. Some comparisons in 1999 are between values obtained in July 1999 (prior to the beginning of ULV applications of malathion for boll weevil reproduction-diapause control in August) with those obtained in October at which time the reproduction-diapause program had ended. All other comparisons are between LC_{50} values obtained in the fall with those obtained in the spring of the same or following year at the same location.

[c] RR_{50} and 95% CL calculated by the formula of Robertson and Priesler (1992); LC_{50} values were considered significantly different if the 95% CL of the resistance ratio at the LC_{50} level did not include 1.0.

Table 2. Mortality of adult tarnished plant bugs exposed to malathion in a glass-vial bioassay. The four collection locations were in Region 2 (south Delta) of the boll weevil eradication program in Mississippi. Eradication began in Region 2 with reproduction-diapause applications of ULV malathion in August of 1998.

Collection location	**Date collected**	***n***	**Slope + SE**[a]	**LC_{50}**	**95% CL**	**Comparison**[b]	**RR_{50}** [c]	**95% CL**
Yazoo city	May 2000	270	0.5 + 0.10	7.2	3.0-11.2	C;	1.5	0.8-2.7
Yazoo City	Nov. 2000	270	0.4 + 0.07	15.6	8.8-23.4	C; May 00: May 01	3.2; 2.2; 2.5	2.0-5.2; 1.1-4.6; 1.5-4.1
Yazoo City	May 2001	150	1.3 + 0.20	6.3	5.1-7.9	C;	1.3	1.0-1.7
Yazoo City	Oct. 2001	240	0.5 + 0.09	77.1	52.1-129.9	C; May 01	15.8; 12.2	10.0-24.9; 7.6-19.6
Louise	May 2000	360	0.5 + 0.06	58.0	42.4-82.2	C;	11.9	8.2-17.1
Louise	Nov. 2000	270	0.5 + 0.08	149.0	109-217.0	C; May 00; May 01	30.5; 2.6; 9.5	20.9-44.5; 1.6-4.1 6.5-13.9
Louise	May 2001	210	1.2 + 0.17	15.7	13.1-18.8	C;	3.2	2.5-4.1
Louise	Oct. 2001	180	1.0 + 0.17	85.9	67.1-116.4	C; May 01	17.6; 5.5	12.9-24.0; 4.0-7.5
Delta City	May 2000	240	0.7 + 0.11	17.1	12.9-21.9	C;	3.5	2.6-4.8
Delta City	Nov. 2000	300	0.7 + 0.08	117.6	91.5-155.7	C; May 00 May 01	24.1; 6.9 10.6	17.6-32.9; 4.8-10.0; 7.6-14.9
Delta City	May 2001	180	1.1 + 0.18	11.1	8.7-13.6	C;	2.3	1.7-3.0
Delta City	Oct. 2001	300	0.6 + 0.07	49.0	37.1-64.5	C; May 01	10.0; 4.4	7.3-13.8; 3.1-6.3
Onward	May 2000	270	0.8 + 0.10	17.9	13.8-6.2	C;	3.7	2.7-4.4
Onward	Nov. 2000	240	1.0 + 0.11	61.6	48.8-76.3	C; May 00 May 01	12.6; 3.4; 9.5	9.5-16.7; 2.5-4.8; 6.4-14.1
Onward	May 2001	210	0.7 + 0.12	6.5	4.3-8.6	C;	1.3	0.9-1.9
Onward	Oct. 2001	300	0.5 + 0.07	43.2	31.2-59.9	C; May 01	8.8; 2.0	6.1-12.7; 1.3-3.1

[a] See footnote a , Table 1.

[b] All LC_{50} values obtained at the four locations were compared to the LC_{50} value obtained for susceptible bugs collected and tested from Crossett, AR in November of 1999. All other comparisons are between LC_{50} values obtained in the fall with those obtained in the spring of the same or following year at the same location.

[c] See footnote c, Table 1.

Table 3. Mortality of adult tarnished plant bugs exposed to malathion in a glass-vial bioassay. The four collection locations were in Region 3 (hill country) of the boll weevil eradication program in Mississippi. Eradication began in Region 3 with reproduction-diapause applications of ULV malathion in August 1997.

Collection location	**Date collected**	***n***	**Slope + SE**[a]	**LC_{50}**	**95% CL**	**Comparison**[b]	**RR_{50}** [c]	**95% CL**
Grenada	May 2000	270	0.7 + 0.10	19.8	15.1-25.2	C; Nov. 00	4.0; 2.8	3.0-5.5; 1.4-5.7
Grenada	Nov. 2000	240	0.5 + 0.08	7.0	2.9-11.7	C; May 01	1.4; 1.1	0.7-2.8; 0.5-2.1
Grenada	May 2001	180	0.9 + 0.15	6.7	5.0-8.6	C;	1.4	1.0-1.9
Grenada	Oct. 2001	210	0.6 + 0.11	8.8	5.0-12.6	C; May 01	1.8; 1.3	1.1-2.9; 0.8-2.2
Winona	May 2000	240	0.6 + 0.12	5.6	2.4-8.6	C;	1.1	0.6-2.1
Winona	Nov. 2000	210	0.6 + 0.13	19.8	12.5-27.1	C; May 00; May 01	4.0; 3.5 2.3	2.7-6.0; 1.8-6.9; 1.5-3.5
Winona	May 2001	180	1.1 + 0.16	8.7	7.0-10.8	C;	1.8	1.4-2.4
Winona	Oct. 2001	180	0.8 + 0.15	23.5	16.4-31.2	C; May 01	4.8; 2.7	3.4-6.8; 1.9-3.9
Oxberry	May 2000	240	1.0 + 0.13	9.3	6.8-11.6	C;	1.9	1.4-2.6
Oxberry	Nov. 2000	240	0.4 + 0.07	10.5	3.9-18.5	C; May 00; May 01	2.2; 1.1; 1.5	1.1-4.4; 0.5-2.4; 0.7-3.2
Oxberry	May 2001	180	1.1 + 0.16	6.9	5.5-8.5	C;	1.4	1.1-1.9
Oxberry	Oct. 2001	180	0.7 + 0.14	10.1	5.9-14.2	C; May 01	2.1;1.5	1.4-3.2; 0.9-2.3
Holcomb	May 2000	210	1.0 + 0.19	5.3	3.0-7.2	C;	1.1	0.7-1.7
Holcomb	Nov. 2000	270	0.6 + 0.09	10.7	6.8-14.7	C; May 00; May 01	2.2; 2.0 2.2	1.5-3.3; 1.2-3.5; 1.4-3.5
Holcomb	May 2001	180	1.1 + 0.17	4.8	3.6-6.0	C;	1.0	0.7-1.3
Holcomb	Oct. 2001	180	0.5 + 0.14	6.1	1.8-9.9	C; May 01	1.3; 1.3	0.6-2.5; 0.6-2.6

[a] See footnote a, Table 1.

[b] See footnote b, Table 2.

[c] See footnote c, Table 1.