DOSE-MORTALITY AND RAINFASTNESS OF ULV MALATHION/COTTONSEED OIL FORMULATIONS FOR THE BOLL WEEVIL

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ABSTRACT

Relationships were established between boll weevil mortality and malathion residues measured on cotton leaf disks before and after applying 2.5 mm of artificial rainfall. Four ratios of malathion to cottonseed oil (1:0, 2:1, 1:1, and 1:2) were applied to the leaf disks using a spinning disk sprayer. Bioassays of 24 and 48 hour mortality of boll weevils placed on leaf disks in petri dishes were compared to chemical assays of malathion on the disks. No significant differences in either 24 or 48 hour mortality were found between the formulations. Data were pooled to obtain LD_{50} and LD_{90} estimates of 1.02 and 2.25 µg malathion per cm^2 of leaf surface, respectively, at 24 hours of exposure and 0.86 and 1.71 µg malathion per cm^2 of leaf surface, respectively, at 48 hours. The artificial rainfall washed most (66-88%) of the malathion residue from leaf surfaces for all four ratios of malathion to cottonseed oil; correspondingly, post-rain mortality was reduced by 48-95%. These data should help boll weevil eradication managers to make decisions about reapplying malathion to fields subjected to rainfall between scheduled applications.

INTRODUCTION

Boll weevil (*Anthonomus grandis* Boheman) pheromone traps in combination with aerially applied ULV malathion (0, 0-dimethyl phosphorodithioate of diethyl mercaptosuccionate) have been the primary tools used for boll weevil eradication. Pheromone traps indicate the location of the populations, and malathion decimates them. When weevil numbers are reduced to very low levels (about 1 weevil per 3 ha), traps may capture enough weevils to make malathion applications unnecessary. $LD_{50's}$ (lethal dose = dose per insect required to kill 50 % of a population) for topically-applied malathion on field-collected boll weevils have been highly variable; however, Martin et al. (1996) reported that no convincing evidence of resistance in the United States has been seen. Insects from the R. T. Gast Insect Rearing Laboratory, Mississippi State, MS were used as the reference colony for their study, and the LD_{50} for malathion (0.586 μg per weevil – 48 hr) was at least as high as that of all but four of the 16 field collections tested. Jones et al. (1998) reported a somewhat higher LD_{50} (1.03 μg per weevil – 48 hr) for weevils of the same colony. Thus, a relationship between boll weevil mortality and topically-applied malathion has been established, but a relationship between boll weevil mortality and malathion residue per unit area of cotton leaf has not been established.

Malathion has been mixed with cottonseed oil (CSO) to increase spray volume for improved

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plant coverage (Mulrooney et al. 1995, Mulrooney 1998a). In a petri dish study using fresh cotton leaves, increased effectiveness of kill occurred when microapplicated droplets of malathion (Cythion RTU, 46.2% AI; American Cyanamid Co., Ag. Div., VPC Ventures, Wayne, NJ) were mixed with CSO (Haynes et al. 1994). Nonetheless, another laboratory study indicated that toxicity was not increased when malathion was mixed with CSO, but toxicity was increased when malathion was mixed with paraffinic oils (Jones et al. 1998). Similarly, field applications of mixtures of malathion and CSO did not appear to increase toxicity over that of malathion alone (Mulrooney 1998b).

Undiluted malathion is readily washed from cotton plants by rain. Rainfall of 12-25 mm caused significant decreases in toxicity of malathion-treated leaves to boll weevils (Hopkins and Taft 1967, Nemec and Adkisson 1969). More recently, as little as 3 mm of rain falling on malathion treated leaves caused 20-40% decreases in boll weevil mortality (Villavaso et al. 1996). Some oil adjuvants including CSO appear to enhance the rainfastness on cotton leaves (Mulrooney 1998a,b).

In the current study, we measured boll weevil mortality versus malathion applied to the surface of cotton leaves and leaf disks. Malathion was tested alone and in combination with three rates of CSO. We also measured the effect of artificial rainfall on malathion residue and mortality of boll weevils exposed to leaves of cotton plants treated with all four formulations.

MATERIALS AND METHODS

Test Insects. Boll weevils were reared from egg to adult at the Gast Insect Rearing Laboratory, USDA, ARS, Mississippi State, MS on the artificial larval diet of Lindig (1979) with Pharmamedia (Trader's Protein, Fort Worth, TX) substituted for cottonseed meal. After emergence as adults, they were fed wax-coated cylinders (8mm [dia.] X 10-40 mm) of the same diet until treatment. Mixed sex, 5-7 day old weevils were used.

Dose-mortality Tests. A Herbi (Micron Corp.; Houston, TX; out of business) rotary sprayer was used to apply malathion to cotton plants and cotton leaf disks. The input voltage to the sprayer was supplied by a 0-20 volts direct current power supply. The supply voltage, and thus the speed of the disc, was varied to produce an array of spray droplets that appeared on oil-sensitive spray cards (TeeJet Spraying Systems Co., Wheaton, IL) to be in the range of those seen on cards collected during a previous study in which ULV malathion was aerially-applied at rates of 0.59, 0.88, 1.17 L per ha (Villavaso et al. 1996). Size and distribution of droplets were not measured. We selected a disk speed that corresponded to a 2.7 volt direct current supply voltage. A range of rates of spray deposit was obtained by varying the speed at which the Herbi sprayer moved over the targets. Sprays were applied at a height of approximately 0.5 m above the targets.

To minimize drift and obtain a reasonably uniform distribution of malathion (FYFANON® ULV 95%; 1.17-kg per liter; CHEMINOVA, Lemvig, Denmark), dose-mortality tests were conducted in a large engineering shop area at Mississippi State University, Mississippi State, MS. Plastic sheeting was used to cover the floor of the engineering shop where malathion was applied. For each application, 12 cotton leaf disks (2 = 3.8 cm, 10 = 3.2 cm diameter) cut from greenhouse cotton plants were arranged with their outer edges almost touching each other on paper towels spread over a 2.5 x 20.3 x 61 cm board placed on the sheeting. The 10 disks used for the bioassays were cut slightly smaller than the 2 chromatographic standards so that they would fit nicely into 3.5 cm petri dishes. The towels were used to absorb malathion droplets not striking the target disks. The 3.8 cm disks were used to estimate the amount of malathion deposited per unit of leaf surface, and the 3.2 cm disks were used in the bioassay. A single pass of the rotary spray over each set of 12 disks was considered to be one application, and a total of 15 applications were made for each of 4 malathion : CSO formulations: 1:0, 2:1, 1:1, and 1:2.

Application speed was varied with each pass of the sprayer to get a range of spray deposits for determining the dose-mortality relationship. After the spray had dried, the 3.8 cm discs were placed in glass jars for subsequent gas chromatographic (GC) analysis. Jars were left open and no condensation was observed. Because weevils had access to both sides of a leaf disk, malathion per unit area of both sides of a disk was calculated. Each 3.2 cm disc was placed in a 3.5 (dia.) X 1.0 cm petri dish labeled according to treatment for bioassay. Petri dishes were taken to the laboratory, and one boll weevil was placed in each dish held at ca. 27°C. Mortality was recorded 24 and 48 hours later. Weevils that were unable to remain upright were considered to be dead.

Chromatograph standards were obtained by applying 100 µL of a mixture of 1 part malathion and 499 parts CSO to each of six 3.8 cm leaf disks. Two untreated control disks were also analyzed to assure that no malathion was present on the leaves before the tests were conducted. The dilution was necessary to obtain amounts of malathion that were of a magnitude similar to that which we expected to capture on the leaf disks. Four droplets of this mixture were applied to the two-3.8 cm GC standard disks with a 25 µl syringe. For both the dose-mortality test and the GC standards, each set of 3.8 cm leaf disks was washed for 1 min. in methanol. A 2 µL aliquot of this rinsate was injected into a Hewlett-Packard 5890A GC with an electron capture detector. The GC had the initial oven temperature set at 150° C. The temperature was elevated 5° C per min. for 4 min. and then 10° C per min. for 5 min. The injector and detector temperatures were 200 and 300° C, respectively. Helium was the carrier gas, and the make-up gas was as 90:10 mixture argon and methane. The column (J&W Scientific Cat. No. 125-5032) was 30 m long with an inside diameter of 0.53 mm. Column gas speed was 35 cm per second.

Rainfastness. Tests of rainfastness were conducted in a greenhouse at the USDA-ARS R. W. Harned Laboratory, Mississippi State, MS. For each of the four malathion formulations, six groups of 15 pre-squaring cotton plants in peat pots were sprayed with one pass of the Herbi sprayer. The foliage of each plant was reduced to two upper, horizontally-oriented leaves immediately before plants were sprayed. The above-mentioned two methods were done in an attempt to obtain malathion depositions as uniform as possible for each of the four formulations. Then, as we did for the dose-mortality tests described above, two 3.8 cm and ten 3.2 cm discs were die-cut from leaves treated with each of the four formulations and from six groups of untreated plants. The two large disks were used for GC analysis of malathion deposition and the 10 small ones for bioassay. GC standard disks, both treated and untreated, were obtained and analyzed exactly as was done for the dose-mortality test.

After leaf disks had been cut from the treated plants, the remaining plants were subjected artificial rainfall with a TK 7.5 flooding nozzle (Spraying Systems Co., Wheaton, IL) at 1.05 kPa. The nozzle was operated with the spray cloud being emitted in the horizontal plane 0.5 m above the plants so that the droplets would free-fall onto the exposed leaf surfaces. Artificial rainfall was applied until 2.5 mm had accumulated in rain gauges. Leaf disks were cut from the rain-treated plants and subjected to the same assays as the malathion treated plants not receiving artificial rainfall. Bioassays and chemical assays were conducted in the same manner as those of the dose-mortality tests. The rainfastness experiment was replicated six times.

Statistical Analysis. Dose-mortality curves were computed using mortalities between 10 and 90% using Sigma Plot 5.0 (SPSS Inc., Chicago, IL). Mortality and rainfastness data were analyzed using the SAS PROC-MIXED procedure (Littell et al. 1996).

RESULTS AND DISCUSSION

Dose-mortality tests. Average recovery of malathion on the 3.8 cm leaf disks used as chromatograph standards was 103.4 ± 18% (SD); thus, we did not consider it necessary to apply a correction factor to the unknown deposits on leaf disks. Trends of either increased of decreased mortality with increasing ratios of malathion:CSO were not evident, and no significant differences between 24 hour mortalities ($P = 0.715$; 3, 54df) or 48 hour mortalities ($P = 0.448$; 3, 54 df) associated with treatments were seen. Consequently, we pooled the data to create the 2 parameter power curves shown in Fig. 1. Equations for the curves are as follows:

$$f = 49.414x^{0.7395} \text{ (24 hour; } r^2 = 0.76)$$
$$f = 56.889x^{0.8533} \text{ (48 hour; } r^2 = 0.68)$$

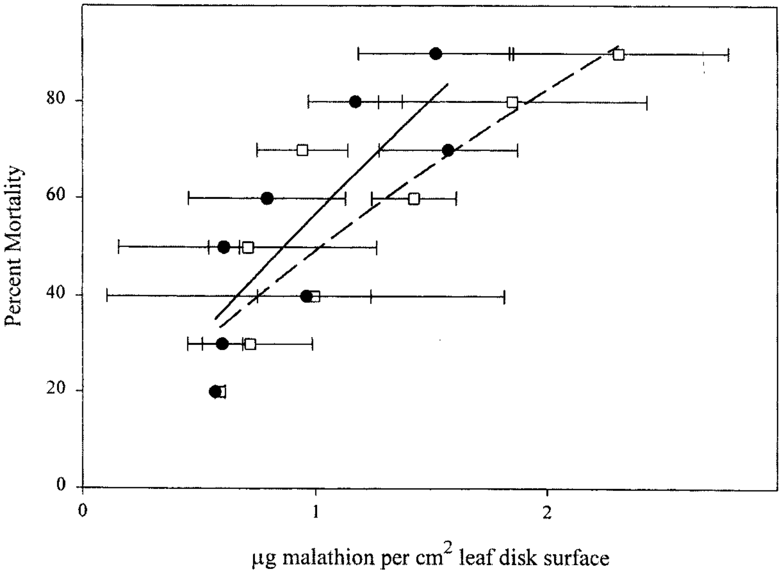

FIG. 1. Percentage Mortality of Boll Weevils 24 Hours (open squares, dashed line) and 48 Hours (solid circles, solid line) After Being Placed on Cotton Leaf Disks Sprayed with a Range of Malathion Dosages

From these equations we calculated LD_{50}'s and $LD_{90's}$ of 1.02 and 2.25 µg per cm^2 of leaf disk surface at 24 hours and 0.86 and 1.71 µg per cm^2 of leaf disk at 48 hours, respectively. These estimates are reasonably close to the LD_{50}'s and $LD_{90's}$ of 0.53 and 1.63 µg per cm^2 of leaf surface at 24 hours and 0.41 and 1.07 µg per cm^2 reported in this supplement (Villavaso et al. 2001). The figures indicate that malathion residues of ca. 2 µg per cm^2 of leaf surface (average of top plus bottom surfaces) should result in significant mortality to boll weevils habitually contacting the surfaces.

The LD_{50} at 48 h after treatment was 0.41 µg per cm^2, and the surface area of each disk was 16.1 cm^2. Thus, leaf disks that resulted 50% mortality at 48 h averaged 6.6 µg malathion per disk. The amount of malathion reported to kill 50% of boll weevils 48 h after *topical* application to individual boll weevils is 0.586 and 1.03 µg per weevil (Martin et al. 1996, and Jones et al. 1998, respectively). In order for a weevil to pick up those amounts of malathion from leaf surfaces, we would expect that much greater amounts must be present on the leaf surfaces. Malathion residues on our leaf disks (6.6 µg per disk) were 6.4 to 11.3 times higher than the reported topical $LD_{50's}$.

Rainfastness. Mean mortality of weevils in the pre-rain bioassays was 100, 93, 87, and 60%, respectively, for the 1:0, 2:1, 1:1, and 1:2 ratios of malathion:CSO ; post-rain mortality was 52, 5, 17, and 5%, respectively (Table 1). Differences in pre-rain mortality were the result of significantly less malathion being deposited on the leaf surfaces at the lower malathion concentrations. Means of µg malathion per cm^2 leaf surface decreased significantly ($P < 0.05$) as malathion concentration in the spray decreased: 6.4 > 4.1> 2.4> 0.4, respectively, for the 1:0, 2:1, 1:1, and 1:2 ratios of malathion:CSO. Post-rain means were reduced to 2.2, 0.5, 0.8, and 0.1 µg malathion per cm^2 leaf surface, respectively, for the 1:0, 2:1, 1:1, and 1:2 ratios of malathion:CSO. The mean of the 1:2 ratio post-rain was significantly less ($P < 0.05$) than that of the other means which were not different from each other. Malathion alone showed less reduction in toxicity following rain than any of the cottonseed oil formulations. More malathion was present to begin with on the malathion alone leaves than on leaves of the other formulations, and the post-rain residues were sufficient for killing half of the weevils tested. Malathion remaining in the cottonseed oil formulations apparently fell below a threshhold needed to maintain significant toxicity. The percent reduction in pre- and post- rain residues among the four treatments were much closer than the bioassay results and not significantly different from each other.

TABLE 1. Percent Mortality of Boll Weevils Exposed to Leaf Disks Treated with 4 Ratios of Malathion to Cottonseed Oil (CSO) and Malathion Residue ± SD on Leaf Disks Before and After 2.5 mm Artificial Rainfall.

	Malathion:CSO							
	1:0		2:1		1:1		1:2	
Rain	% M[a]	Residue[b]	% M	Residue[b]	% M	Residue[b]	% M	Residue[b]
Before	100 AW	6.4 aw	93 AX	4.1 Ax	87 AY	2.4 ay	60 AZ	0.4 az
After	52 BX	2.2 bx	5 BY	0.5 By	17 BY	0.8 by	5 BY	0.1 by
% Red.[c]	48	66	95	88	80	67	92	75

[a] % Mortality; average of 6 replications; Residue average of 4 replications for 1:0, 6 all others.
[b] µg malathion per cm^2 leaf surface
[c] % Reduction
For columns, means followed by different upper case A, B letters significantly different at $P < 0.01$, by different lower case a, b letters at 0.05 (GLM).
For rows, mortality means followed by different upper case W, X, Y, Z letters significantly different at $P < 0.01$. Residue means followed by different lower case w, x, y, z letters significantly different at $P < 0.05$ (GLM).

Toxicity of leaves bioassayed after being subjected to a very small amount of artificial rainfall (2.5 mm) was significantly lower than the pre-rain toxicity for all 4 malathion spray concentrations. Post-rain residues were reduced by 66-88%; correspondingly, post-rain mortalities were reduced by 48-95%, respectively (Table 1).

In summary, we have determined that boll weevils exposed to residues of 1.02 and 2.25 μg malathion per cm^2 leaf surface will result in 50 and 90% kill of those weevils at 24 hours, and 086 and 1.71 μg malathion per cm^2 leaf surface area will result in 50 and 90% kill, respectively, at 48 hours. Rain of as little 2.5 mm will wash away at least 66% of malathion residue. Boll weevil eradication managers should be aware of the rain effect on malathion and treat fields as soon after rainfall as possible.

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