COLOR THE BOLL WEEVIL RED: PENETRATING THE BODY WALL WITH SPRAY DILUENTS

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Abstract

A technique was developed to test the ability of oil spray diluents to penetrate the body wall of adult boll weevils. The dye, Calgo Oil Red N-1700® was used as an indicator that the oil treatments had penetrated the insect's cuticle. The technique consists of (1) applying the oil plus dye to the boll weevil by a dipping process, (2) washing the boll weevil in acetone several times to remove the dye from the outer surface of its body and (3) crushing the weevil using paper chromatography to determine if dye had penetrated the body wall. Most of the tested diluents had excellent penetrating effects. The malathion ulv, which is the technical material and an oil, had a 50% penetration ability. In combination with cottonseed oil and paraffinic oils it had a range of 92 to 100% penetration. Additionally a difference was observed in the penetration properties of different production batches of once refined cottonseed oil.

Introduction

Ultra low volume (ULV) spray technology for aerial pesticide application is an oil based technology. Evaporation of the spray drops during application is the prime factor for this. The Boll Weevil Eradication Programs utilize this technology as a main element of their procedure. This makes eradication economically possible. Developments in reducing malathion concentration (Jones et al. 1998) while maintaining spray coverage will require oil diluents. This includes developing other insecticides for ULV application (Mulrooney, 1998 and Reed et al. 1998). These oil diluents can either enhance or detract from the activity of the insecticides (Jones et al. 1998 and Mulrooney, 1998). Work by Ahmed and Gardiner (1967) showed this enhancement of malathion with kerosene. Their further experiments (Ahmed and Gardiner, 1968) showed penetration of the body wall was the factor for this enhancement. This work demonstrated a need for a simple test for the enhancement of body wall penetration. The number of diluents and combinations available is large. These need to be screened to determine and study the best combination for control use. The technique developed and tested uses Calgo Oil Red N-1700® dye in various diluents and mixtures. This oil sensitive dye has a long history as a marker for USDA ARS Gast Lab reared boll weevils (McKibben et al. 1971). The boll weevil adults were dipped in these dyed mixtures and tested for body wall penetration.

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Materials and Methods

The materials tested for ability to penetrate the boll weevil body wall are listed in Table 1. These were tested alone or in combination with the others. The oil based materials are as follows: malathion ulv (95%, Cheminova-Denmark); once refined cottonseed oil (1996 and 1997 products from same plant - chemical extraction); Orchex 796® (paraffinic horticulture oil, Exxon); WS2908 (research material of Orchex 796 and polymers for drift control); HM9737 (research material, processed seed oil emulsifier); commercial vegetable spray oil (processed oil with emulsifiers for water mixes). A general laboratory grade acetone was also used as an emulsifying agent. All materials and mixtures were measured and mixed for a total quantity of 20 ml in wide mouth vials. Seventy µg of Calgo Oil Red N-1700®, an oil sensitive dye was added and mixed in each vial.

Boll weevil adults were obtained from field collected infested cotton squares. These adults were held in ½ pint, cardboard cartons at room temperature for 7 days. They were fed boll weevil diet pellets from the USDA ARS Gast Lab. With maturity at seven days of age (Teague et al.1983) these adult weevils were sorted into 14 equal sized groups. Each group of 8 or 10 weevils per treatment was utilized that day. The weevils were individually dipped in their treatment. This was done using a fine tweezers. The tweezers were positioned above and in front of the weevil so that the snout was firmly gripped just in front of the head. The weevil was then dipped into the oil up to but excluding its prothorax and head. It was immediately removed and placed on white filter paper in a petri dish.

In order to remove the oil and dye from the exterior surfaces of the weevils the following wash procedure was done. Five minutes after the last dipped weevil of a group was placed to dry, all weevils were placed into a 50 ml beaker with 10 ml of acetone. This beaker with weevils was gently swirled for 20 seconds and all was poured into a filter paper lined funnel. The weevils were then placed on new filter paper in a petri dish to dry. This wash procedure was repeated a second time. After drying the elytra and wings of each weevil were gently opened. Using a scissors these were then clipped off, careful to leave at least $1/10^{th}$ their length. With the abdomen thus exposed three more acetone washes were done. New filter paper and acetone were used with each wash. The filter paper on which the weevils dried was examined each time for red dye. The wash was considered complete when no red dye appeared on the filter paper. Since this generally occurred with the second wash the other washes gave assurance.

A series of 1 dram shell vials was set up in racks for each group by treatment. Into each vial a washed and completely dry weevil was placed. The vial was filled ¼ full with acetone. Using a clean glass rod the weevil was crushed completely. A piece of chromatography paper (1/4 x 2

inches) was placed in each vial of acetone and crushed weevil. The rack of vials was labeled and set under an exhaust hood for approximately 24 hours. The chromatography paper was examined for red bands with results recorded. These procedures were followed with a group for each treatment completed on a given day. This was repeated on five separate dates for each treatment.

Results and Discussion

The comparative results of these tests are presented in Table 1. This technique appears to work for screening the ability of each of these oil based materials to penetrate the body wall of the adult boll weevil. The results of Jones et al. (1998) showed a greatly enhanced toxicity of malathion ulv on boll weevils when mixed with Orchex 796®. The 100% penetration of this paraffinic oil alone and in treatments 8 and 12 versus 50% for malathion ulv by itself explains this. The work of Ahmed and Gardiner (1967 and 1968) further support these conclusions. The vegetable oils offer a more complex situation. If both the 1996 and 1997 production of once refined cottonseed oil gave the same results, then the emulsifier in the commercial vegetable oil spray could be the difference. However, the two different productions of the cottonseed oil differ in penetration ability. The 1997 production by itself was 100% but with malathion was 92%. The 1996 production penetrated 84% of the time. These results are supported by the topical toxicity results of Jones et al. (1998). That work indicated that the cottonseed oil was detrimental to the malathion control efficacy for boll weevil.

The results of this study appear to show the same for the 1997 production and possibly worst for the 1996 production which was used in the Jones et al. (1998) study. This indicates that if cottonseed oil is to be used as a diluent for insecticide applications there should be a set of industry standards developed.

This may be needed for all vegetable oils used as diluents. This technique was developed as a simple comparison tool. For more scientific studies there are questions about the penetration ability of acetone that were not answered and may have affected the results. The variation in results, however, indicates that it can serve as a simple screening tool.

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Table 1. Penetration of Calgo Oil Red N-1700® Dye Through the Boll Weevil Cuticle.

Treatment*	Mixture	Number Treated	% Penetration
1 CS 1997	Undiluted	48	100%
2 O	Undiluted	48	100%
3 WS	Undiluted	48	100%
4 O + HM	10ml + 10ml	48	100%
5 WS + HM	10ml + 10ml	48	100%
6 M	Undiluted	48	50%
7 M + O +HM	5.4ml +7.3ml +7.3ml	48	98%
8 M + WS + HM	5.4ml + 7.3ml +7.3ml	48	100%
9 M + CS 1997	3.1ml + 16.9ml	48	92%
10 HM	Undiluted	48	98%
11 O + A	17.8ml + 2.2ml	48	100%
12 M + O + A	2ml + 16ml + 2ml	48	100%
13 CS 1996	Undiluted	50	84%
14 VO	Undiluted	50	86%

* Cottonseed Oil (CS), Orchex 796® (O), WS2908 (WS), HM9737 (HM), Malathion ULV (M), Acetone (A), Vegetable Oil Spray (VO).