PATTERNS OF EFFICACY OF BOLL WEEVIL INSECTICIDES USING A LEAF DISK BIOASSAY

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Abstract

Observations in field trials of boll weevil insecticides indicated a need to investigate patterns of efficacy by boll weevil insecticides at different times after treatment application and for selected exposure times. Laboratory bioassays were conducted in which adult boll weevils were exposed to leaf disks cut from leaves collected from treated plants. These studies investigated patterns of mortality caused by exposure of boll weevils for 6, 12, 24, 48, or 72 h to fipronil, azinphosmethyl, two rates of endosulfan, oxamyl, and a low rate of ULV malathion, at time intervals of 0, 24, 48, and 72 h after treatment application. Mortality continued to increase between observations at 24 to 72 h after initial exposure regardless of duration of the exposure or the insecticide used. However, changes in mortality between observation times were greater for Fipronil, azinphosmethyl, and the high rate of endosulfan than for other materials. When duration of exposure was <12 h mortality was typically reduced, except when initial exposure to insecticides was ≥48 h after application. No material supplied a high level of mortality when initial exposure was at ≥48 h after treatment application. Fipronil, azinphosmethyl, and the high rate of endosulfan supplied high levels of mortality immediately after treatment application, but fipronil demonstrated greater activity than other materials when initial exposure was delayed for 24 h. These results illustrate that consideration of both residual activity and duration of exposure can increase both quality and quantity of information yielded by bioassays.

Introduction

Results of field efficacy trials for control of boll weevils indicated a need to further investigate relationships between observed mortality, residual activity, and time of exposure to insecticides. In studies reported elsewhere in these Proceedings, insecticide treatments applied at weekly intervals failed to reverse boll weevil population increases in small plots, but treatments applied at 3-d intervals resulted in rapid reductions of population levels. Associated observations indicated as many as 60% of adult boll weevils were in protected situations, such as within the bracts of squares, when insecticides were applied. Thus, we surmised the observed lack of control may have resulted from limited exposure time to treated surfaces as much as inadequate residual activity of applied materials. To investigate this possibility we designed a bioassay to separate the effects of initial toxicity, residual activity, and duration of exposure to selected insecticides on observed mortality.

Materials and Methods

Only native adult boll weevils obtained either by field collection or from traps serviced twice daily were used in the bioassays. The weevils were held in the laboratory at about 75°F and under continuous light, and were fed fresh squares and 10% sucrose solution for 2-3 days before use. Weevils of mixed sex were used in the order of their collection to avoid large discrepancies in weevil age among replications of the experiment.

Insecticide treatments were endosulfan (Phaser 3E; 0.375 and 0.5 lb. a.i./acre), azinphosmethyl (Guthion 2L; 0.25 lb. a.i./acre), oxamyl (Vydate 3.77 CLV; 0.25 lb. a.i./acre), fipronil (Regent 2.5 EC; 0.05 lb. a.i./acre), and an untreated check. ULV malathion was also included, but a malfunction of the "Microfit" controlled-droplet applicator (Micron Sprayers Ltd., Bromyard, Herefordshire, U.K.) resulted in a rate of 5.1 oz. of technical material/acre. Treatments were randomly assigned to single-row plots, 10 ft in length, that were separated by 4 untreated buffer rows, except for the ULV malathion treatment, which was applied to the northern-most row and was separated from other treatments by 6 untreated rows to avoid drift of the material onto the other treated rows. Materials other than ULV malathion were applied using a CO_2 pressurized backpack sprayer equipped with three TX5 hollow cone nozzles (one over the top, two on drops) delivering 10 gal./acre of spray mixture at 40 psi. The experiment was repeated 3 times, with treatments applied on 11, 22, and 26 July. A previously untreated section of row was used in each new replication.

Effects of insecticides were assessed by exposing adult boll weevils to treated leaf surfaces at 0, 24, 48, and 72 h after treatment application. Within each post-treatment time, exposure durations of 6, 12, 24, 48, and 72 h were examined. Thus, a total of 140 treatment combinations (7 insecticide treatments × 4 post-treatment exposure times × 5 exposure durations) were used in each replication. Bioassays were performed using "Falcon 3046" 6-well tissue culture plates with wells approximately 1.4 in. in diameter and 0.75 in. deep. One bioassay plate was assigned to each treatment combination within a replication. A disk of filter paper was placed in the bottom of each well and wetted with 100 μl of distilled water to maintain a high humidity in the well. The treated leaf disk, also about 1.4 in. in diameter, was placed on top of the filter paper disk. A single tissue culture plate containing one adult weevil in each cell constituted the experimental unit. Upper, fully expanded leaves were collected from the treatments at

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designated times, sealed in plastic bags, and immediately carried to the laboratory where disks were cut and installed in the tissue culture plates. After leaf disks and weevils were installed, the edges of the plates were sealed with parafilm to prevent drying of the leaf disks. Except for treatment combinations using an exposure duration of 72 h, the treated leaf disks were replaced with freshly collected untreated disks after the assigned exposure time had elapsed. Tissue culture plates were held in an environmental chamber at 85°F and continuous light. Mortality was assessed at 24, 48, and 72 h after initial exposure.

Because mortality estimates represented repeated observations of individual experimental units, the data were analyzed by repeated measures analysis using the SAS procedure PROC GLM and the REPEATED statement (SAS Institute, 1988). A test of sphericity was used to determine if the data satisfied the Huynh-Feldt condition. When the Huynh-Feldt condition was accepted ($P>0.05$), all data were assessed using the usual univariate F tests. When the Huynh-Feldt condition was rejected at $0.05>P>0.0001$, the Greenhouse-Geisser epsilon adjusted probability (G-G adjusted P) was used for hypothesis testing involving repeated effects. When the Huynh-Feldt condition was rejected at $P\leq0.0001$, multivariate tests (Wilks' Lambda) were used to assess repeated factors and their associated interactions.

Results and Discussion

Estimated mortality was dependent upon time of observation; mortality continued to increase between 24 and 72 h after initial exposure of weevils to the treated leaves (Wilks' Lambda=0.486; F=185.28; df=2, 351; P=0.0001). The extent of the change in mortality with increased time after initial exposure was variable among treatments (Wilks' Lambda=0.904; F=3.04; df=12, 702; P=0.0003). Comparisons among treatments when the data were considered over post-treatment exposure times and durations of exposure indicated that differences in mortality among observation times tended to be greater for fipronil, azinphosmethyl, and the high rate of endosulfan than for other treatments (Fig. 1). Differences in mortality among observation times also varied among the different post-treatment exposure times (Wilks' Lambda=0.772; F=16.13; df=6, 702; P=0.0001). Comparisons among post-treatment times of initial exposure, when the data were considered over insecticidal treatment and duration of exposure, indicated that differences among observation times were greatest when weevils were exposed immediately after treatment application (Fig. 2). These differences tended to diminish with increasing post-treatment time of exposure. The reduction in differences among observation times with increasing post-treatment time of exposure probably resulted from the greatly diminished toxicity of the treated surfaces at the later exposure times. Because mortality tended to be greater, and probably more indicative of insecticidal efficacy, at later observation times, unless stated otherwise subsequent discussions of results in this report refer to mortality observed at 72 h after initial exposure of weevils to the treated leaves.

Duration of exposure significantly influenced mortality (F=3.55; df=4, 352; P=0.0074). In general, exposure periods of ≥12 h duration resulted in increased mortality. However, differences in mortality among times of observation did not appear to be affected by the duration of exposure (Wilks' Lambda=0.981; F=0.85; df=8, 702; P=0.5565); mortality at each duration of exposure increased similarly with increasing time of observation (Fig. 3). Effects of duration of exposure on mortality were dependent on the post-treatment time of initial exposure (F=1.86; df=12, 352; P=0.0380). When weevils were exposed to treated leaves immediately after treatment application, differences associated with duration of exposure were minimized (Fig. 4). When weevils were exposed to leaves treated 24 h earlier, longer exposure times resulted in increased mortality. At post-treatment times ≥48 h, mortality was generally reduced and no differences were detected among exposure times.

The significant insecticide by post-treatment time of exposure interaction (F=7.84; df=18, 352; P=0.0001) indicated differences in residual activity among insecticides tested. When the data were considered over duration of exposure, only fipronil, azinphosmethyl, and the high rate of endosulfan supplied a high level of efficacy against boll weevils when weevils were exposed to treated leaves immediately after exposure (Fig. 5). When initial exposure occurred 24 h after treatment application, fipronil supplied greater mortality than any other insecticide. Although fipronil did not supply high levels of mortality when weevils were exposed at 48 or 72 h after treatment application, it was the only material that resulted in mortality greater than that observed in the untreated check. Oxamyl and ULV malathion appeared less sensitive to post-treatment time of exposure than other materials, but neither material was highly efficacious at any exposure time. However, ULV malathion was applied at less than half the commonly used rate of 12 oz./acre. The observed lack of sensitivity of ULV malathion to post-treatment time of exposure may not be indicative of the response at normal use rates.

In general, only fipronil, azinphosmethyl, and the high rate of endosulfan provided high levels of efficacy against boll weevils under any conditions. Fipronil showed greater residual activity than other materials, but no material supplied efficacious control of the boll weevil at >48 h after treatment application. Both duration of exposure of weevils to treated leaves and the time after exposure at which mortality was observed influenced efficacy. These results indicate that poor efficacy observed against an increasing boll weevil population in field tests may have been caused by a combination of inadequate residual activity of the

toxicants and failure of weevils residing in protected sites to receive adequate exposure to the toxicants. We suggest that because our bioassay accounts for variations in time of post-treatment exposure, duration of exposure, and time of occurrence of mortality, our design will yield better insight into potential field effectiveness of assayed materials than bioassays that use a fixed or unlimited duration of exposure, or that assess mortality at a single, fixed time after exposure (e.g., Jones *et al.* 1996, Mulrooney *et al.* 1995).

References

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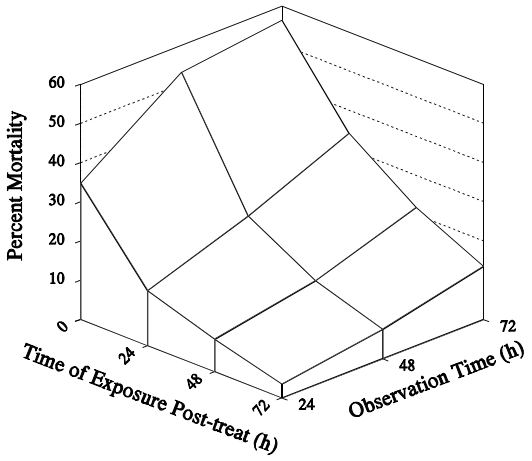

Fig. 2. Mortality of boll weevils exposed to insecticides at different times after treatment application. The data were pooled over insecticides and duration of exposure.

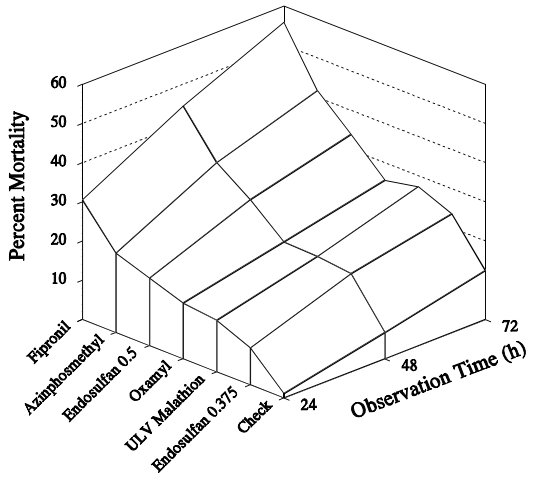

Fig. 1. Mortality of boll weevils at various times after initial exposure to insecticides. The data were pooled over post-treatment time of exposure and exposure duration.

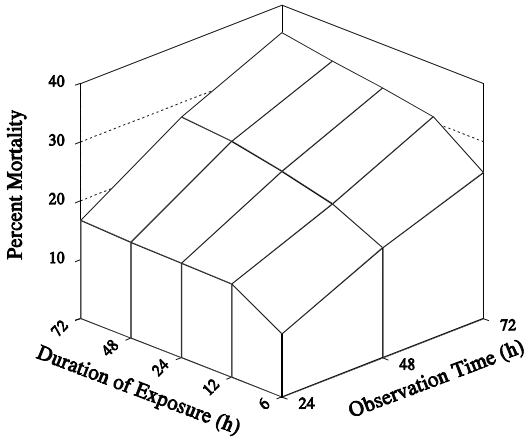

Fig. 3. Mortality of boll weevils exposed to insecticides for different durations. The data were pooled over insecticides and time of exposure after treatment application.

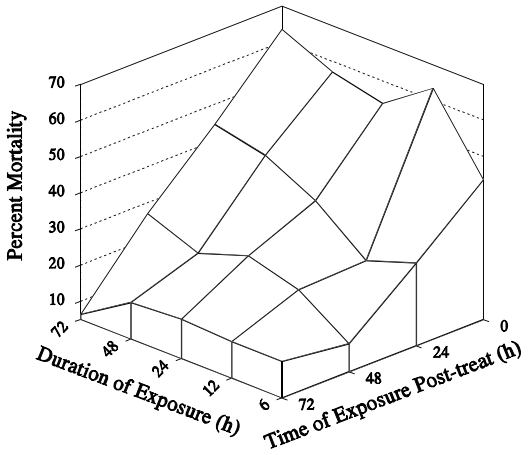

Fig. 4. Mortality of boll weevils exposed to insecticides at different times after treatment application and for various durations of exposure. The data were pooled over insecticides and mortality was assessed at 72 h after initial exposure.

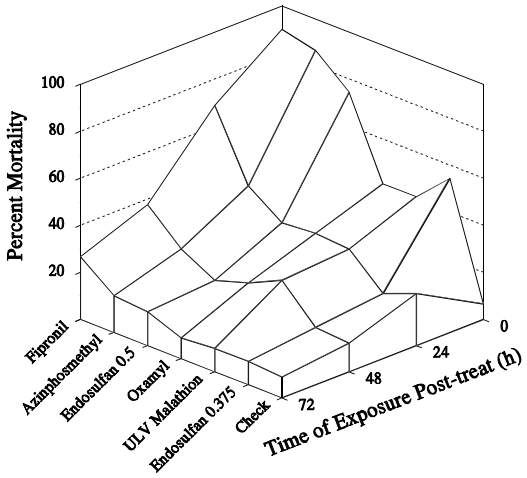

Fig. 5. Mortality of boll weevils exposed to various insecticides at different times after treatment application. The data were pooled over duration of exposure and mortality was assessed at 72 h after initial exposure.