NEW INSIGHTS REGARDING ESTIMATING LYGUS SUSCEPTIBILITY TO INSECTICIDES

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Abstract

Lygus susceptibility was found to vary widely from year to year, from region to region and, for some insecticides, even within the season. It is for this reason that producers need current, region-specific recommendations in order to determine which insecticides are most effective at their locale. Our studies were intended to improve understanding of the reliability of glass vial bioassays for estimating efficacy of insecticides used against lygus bugs. Results show that the standard glass vial method offers considerable promise for detecting differences in susceptibility of lygus to some, but not all, insecticides. However, mortality in vial bioassays did not serve as a reliable predictor of the relative toxicity of residues of five insecticides in field treatments. Therefore, field evaluations of insecticide efficacy continue to be essential for selecting the insecticides that provide the best control of lygus. Once the most effective materials are selected from field trial results, bioassays can be used to efficiently monitor changes in population susceptibility to these insecticides. Additional new insights provided by our studies are that efficacy of residues of insecticides declined rapidly, such that after three days all insecticides caused very little mortality to adult lygus bugs. Lastly, we found a marked difference between residual and direct contact toxicity of the five insecticides evaluated. Even the insecticide treatments that resulted in relatively low toxicity in residual exposure tests killed 95-100% of lygus bugs that they contacted directly under field conditions. This finding indicates that producers experiencing severe problems with lygus control would be well advised to improve insecticide coverage.

Introduction

Lygus bugs are very serious pests of cotton in the desert Southwest, causing losses estimated at $25.25 per acre in Arizona in 1996 (Hardee and Herzog 1997). Feeding by lygus reduces cotton yields due to shedding of immature squares and damage to bolls (Wene and Sheets, 1994). Though lygus cause losses of over $7,000,000 each year to Arizona cotton, and reports of poor insecticidal control of lygus are commonplace, growers continue to combat this pest with essentially the same approaches used over the past two decades. Until such time as there are new breakthroughs in the technology used for controlling lygus bugs, reducing losses caused by this pest to cotton will hinge on utilizing existing methods more efficiently. For this reason the University of Arizona Cotton IPM program has redoubled educational efforts focused on improving use of lygus sampling methods and thresholds. In conjunction with this effort, we have striven to provide growers with the information necessary to avoid using ineffective insecticides and to limit problems associated with development of lygus resistance to insecticides.

When successful, field trials are the most reliable approach to estimating efficacy of insecticides against lygus bugs. However, they are expensive to conduct, require large treated and untreated plots, lygus are notoriously difficult to predict where and when populations will build up, and, due to movement of lygus adults, populations frequently decline in control plots, renderring trial results all but worthless. Laboratory-based bioassays of susceptibility to insecticides offer economic and precision advantages over conventional field evaluations of insecticide performance. However, the less expensive and more precise result of bioassays is useful only if the information obtained accurately reflects real-world differences in field performance of insecticides.

Because there is little published information regarding the degree to which lygus bioassays reflect the activity of insecticides under field conditions, in 1997 we investigated this question for the glass vial bioassay and five insecticides. We evaluated susceptibility of two lygus populations to: 1) glass vial bioassays; 2) field-applied, field-weathered residues on cotton leaves; and, 3) direct, topical application of dilute insecticide in the field. We present these new findings in concert with conclusions from previous work demonstrating that lygus bugs can vary greatly in susceptibility to insecticides both regionally and locally. This project comprised a four-year collaboration of the UA-Extension Arthropod Resistance Management Laboratory and the Arizona Cotton Research and Protection Council.

Methods

Locations Sampled

During the 1994, 1995, and 1996 seasons, lygus bugs were collected on a statewide basis (Fig. 1) from up to 22 locations throughout the cotton-producing regions of Arizona (Dennehy and Russell 1996, Russell et al. 1997). Collections were made from July through October and all populations were bioassayed for susceptibility to bifenthrin (Capture®) and acephate (Orthene®). At two locations, Casa Grande and Marana, sampling of lygus bugs was conducted on a monthly basis from 1995 through 1997 in order to estimate temporal variation in population susceptibility to bifenthrin and acephate. In 1997, studies focused on characterizing differences in response of a representative

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multiple-resistant population from Central Arizona (Maricopa) and a comparatively susceptible population from Eastern Arizona (Safford). Lygus from these two locations were collected from July through October, 1997, and transported to the EARML facilities in Tucson where susceptibility was estimated using glass vial bioassays or bioassays employing field-weathered insecticide residues. Additional collections of Safford and Maricopa lygus were made in September, 1997, to estimate in a field trial the direct toxicity (rather than toxicity of residues) of insecticides to the Maricopa and Safford lygus.

Collection of Lygus

Lygus populations were sampled from alfalfa fields located adjacent to cotton fields. Using sweep nets, approximately 400-600 adult lygus bugs were collected from each location. Bugs were emptied from the sweep nets into lunch-size paper bags within which a base of alfalfa cuttings had first been placed. These bags were then placed over ice within ice chests and transported to the laboratory in Tucson. In the laboratory, most lygus were tested within 24 hours. When necessary, they were maintained on fresh alfalfa cuttings in one-quart plastic containers, for 24-48 hours at 60-68° F (15-20° C), prior to being tested.

Glass Vial Bioassay Method

We used the glass vial bioassay technique described by Knabke and Staetz (1991). Modifications we made to this technique included: drying treated vials on a commercial hot dog warmer, covering infested vials with dialysis membrane instead of screw caps, and the elimination of carbon dioxide for anesthetizing. Standard 0.67 ounce (20 ml), screw-cap scintillation vials were used. A volume of 0.16 ounces (0.5 ml) of insecticide solution was placed in each vial. Vials were immediately placed on the hot dog warmer, operating at room temperature, and slowly rotated until solutions dried completely. This provided thorough coverage of insecticide on the inner surface of the vials.

Bioassays for the statewide surveys of susceptibility to bifenthrin and acephate conducted in 1994-96 used technical insecticide diluted in acetone. Contrasts of Maricopa and Safford lygus bugs were conducted in 1997 with the following formulated insecticides: acephate (Orthene 80S®), endosulfan (Gowan endosulfan 3EC®), imidacloprid (Provado 1.6F®), methamidophos (Monitor 4S®), and oxamyl (Vydate L®). All solutions were formulated on the basis of weight of active ingredient insecticide to total volume of solution. Imidacloprid solutions were prepared with distilled water, owing to problems with solubility in acetone; acetone was used as the diluent for all other insecticide solutions.

Five adult lygus were aspirated into each vial. Vials were then closed with 1Ó x 1Ó squares of dialysis membrane secured with a #8 rubber band. Vials were held for 3 hours in an incubator maintained at 80°F (27 ± 2°C), after which mortality was recorded. Individuals unable to exhibit repetitive movement of locomotory appendages were scored as dead. Subjects unable to walk one body length but exhibiting repetitive movement were scored as moribund. Live individuals walked at least one body length. Mortality values reported herein represent only the individuals scored as dead. Inclusion of moribund individuals in mortality estimates did not alter our results appreciably. Statistical significance of differences between the populations evaluated was determined by ANOVA of mean mortality values, transformed with arcsin$\sqrt{x}$.

Field-Weathered Residues

Applications of five insecticides, acephate (Orthene 80S®), endosulfan (Gowan Endosulfan 3EC®), imidacloprid (Provado 1.6F®), methamidophos (Monitor 4S®), and oxamyl (Vydate L®) were made in July and August, 1997, to a cotton field located near Maricopa, Arizona, using commercial ground spray equipment. Treated plots were 18 rows wide, on 38-40 inch centers, running the length of each field. Treatments were made using a high clearance John Deere 6500 Hi Cycle ground sprayer with Raven SCS 750 three module chemical injection system. Treatment sprays were made approximately 12 inches above canopy height using flat fan nozzles delivering insecticide solutions of 15 gallons per acre at 40 lbs. per square inch. Ten leaves from the fifth main-stem-node position were collected from the central portion of each treatment plot on each sampling date. Samples were collected immediately after spray materials dried, 1 day, and 3 days after treatment. Toxicity of these residues to lygus bugs was estimated using the cell bioassay method.

The cell bioassay method was modified from a procedure described in Dennehy et al. (1993). Cotton leaves collected from field trials were sandwiched between plates of Plexiglass plastic that created a holding cell on the leaf surface. The three plastic plates were rectangles measuring 4 x 3 x 0.3 inches.. The holding cell was formed by 1.7 inch holes centered in the top two plates. The botton plate, over which the leaf was placed had an offset 0.8 inch hole through which the leaf petiole extended into water. A layer of pharmaceutical cotton fiber was placed under the leaf to protect it from compression when the assembled plates were bundled together with two rubber bands. A piece of dialysis tubing (Spectra/Pro® molecularporus membrane) was taped over the hole in the top plate.

For each date on which samples of treated leaves were collected, cell bioassays were replicated at least six times for each treatment, including controls. Infested bioassays were held at 79°C (26°C ±2) for 24 hours, after which mortality was recorded. Mortality was assessed using the same criteria as for the glass vial bioassay.

Direct Contact Toxicity

Susceptibility of adult lygus bugs to direct, topical contact of insecticides was estimated in a field trial conducted in September, 1997, at Marana, Arizona. We devised a simple

apparatus that held groups of chilled lygus bugs at the top of the plant canopy while spray equipment treated them. Eight-ounce waxed cups were taped to the top of wooden stakes four feet in length. These stakes+cups were then placed in pairs of adjacent cotton rows, four yards apart and mid-furrow, and were driven into the ground to place the cups at appproximately the top of the canopy. Approximately 4 ounces of ice was placed in each cup.

Three hours prior to making applications in the field, adult lygus collected from Safford and Maricopa were aspirated into eight-ounce wax paper cups, 25-30 per cup. Lids were secured on the cups and they were placed on ice within ice chests and transported to the field. In the field these cups containing chilled lygus were fitted within the cups on stakes containing ice. Therefore, the lygus were being held over ice in the field. Lids were removed from the cups containing lygus just ahead of the oncoming spray equipment and were replaced immediately after treatment. Thereafter, the treated lygus were removed from the field and transferred immediately to clean eight-ounce containers into which a piece of green bean had been placed as a food source. Lygus were transported to the laboratory where they were held at 80°F (27°C) for 24h, after which mortality was recorded in the manner noted above for vial bioassays. For each of the five insecticides and control (water+adjuvant) treatments a total of six replicates (cups) of 25-30 lygus were tested for each of Safford and Maricopa collections. Controls were subdivided into groups that were sprayed with water+adjuvant and groups that were not sprayed. For this latter group, lids on the cups containing lygus were not removed at the time of spraying.

Applications were made using a high clearance John Deere 6500 Hi Cycle ground sprayer with Raven SCS 750 three module chemical injection system. The five insecticides and corresponding rates evaluated were: acephate (Orthene 80S® 1 lb./acre), endosulfan (Thiodan® 32 oz./acre), imidacloprid (Provado 1.6F® 3.75 oz./acre), methamidophos (Monitor 4S™ 32 oz./acre), and oxamyl (Vydate L® 32 oz./acre). The check plot was treated only with water and adjuvant (Bayfolan® 1 qt/100 gal). Treatment sprays were made approximately 12 inches above canopy height using flat fan nozzles delivering insecticide solutions of 15 gallons per acre at 40 lbs. per square inch.

Results and Discussion

Between-Season Changes In Lygus Susceptibility To Insecticides

Statewide surveys from 1994 to 1995 revealed a significant decrease in susceptibility to Capture and Orthene in lygus bug populations throughout Arizona. This corresponded with severe problems with whitefly resistance to pyrethroids and the related high levels of insecticide use in the 1995 season.

In 1996, lygus bugs regained susceptibility to Capture and Orthene at all but one location (Fig 2a,b). This reduction in resistance was attributed to reduced insecticide use stemming from emergency registration of two insect growth regulators for use against whiteflies in Arizona cotton. These findings illustrate that lygus resistance levels can change from year to year, for the better or the worse.

Within-Season Changes in Lygus Susceptibility

Within-season changes in susceptibility to bifenthrin (Fig. 3a) were considerably less than changes in susceptibility to acephate (Fig. 3b). Mean mortality observed with specific concentrations was as little as 5-10% for bifenthrin or as much as 50% or more for acephate. If such differences reflect equivalent changes in the susceptibility of field populations, then growers in areas like Marana are likely to experience highly variable results during the year when using these insecticides. Further correlation of laboratory and field results will be necessary determine if this is the case. We hypothesized that mixing of populations from different crop and non-crop sources is an important mechanism underlying such within-season fluctuations in lygus susceptibility to acephate.

Glass Vial Bioassays

Vial bioassays detected clear differences in susceptibility of Central Arizona versus Eastern Arizona lygus to all five insecticides evaluated. The Central Arizona population was significantly less susceptible to all compounds tested. (Fig. 4a). The order of relative toxicity of compounds was different for Central Arizona and Eastern Arizona. However, in both cases toxicity in vial bioassays of acephate was low while that of its bioactivated product, methamidophos, was highest of the compounds evaluated (Fig. 4a,b).

Field Weathered Residues

Activity of residues against adult lygus dropped precipitously in the first three days following treatment (Fig. 5a), irrespective of how toxic the compounds were in glass vial bioassays. Toxicity of residues at 1-Day after treatment was much higher against the Eastern Arizona than Central Arizona lygus for all compounds except Provado (Fig. 5b). This was very consistent with results of the vial bioassays for Orthene, Vydate, Monitor and Thiodan. In the case of Provado, the vial bioassay did not relate well to toxicity of field residues. Toxicity of 0-Day residues of Central Arizona lygus was very similar for Orthene, Provado, Monitor and Vydate (Fig. 5a). Therefore, differences in relative toxicity in vial assays were not reflected equivalently in toxicity of field residues.

Despite relatively low toxicity in glass vials, in the field acephate residues were at least as toxic as the other insecticides at Day-0 (Fig. 5a). Conversely, Monitor, which was especially toxic in vial bioassays (Figs. 4a,b), was not concomitantly as toxic in the field treatments (Figs. 5a,b).

Direct Contact

All insecticides sprayed directly on lygus adults were very toxic, killing 95-100% (Fig. 6). This outcome was dramatically different from results of both glass vial bioassays and field-weathered residues of the same insecticides. Our results indicate that residual contact bioassays like the vial method greatly underestimate toxicity of insecticides to that proportion of lygus that are contacted directly at the time of treatment. The probability of a pest encountering direct contact, such as we simulated in our trial, will vary widely depending on whether air or ground applications are made and depending on other application parameters such as spray volume, ground speed and nozzle arrangements. Clearly one strong inferrence from this test was that measures that increase spray coverage should increase the probability of directly contacting adult lygus bugs and therein greatly increase efficacy of these insecticides.

Conclusions

Studies conducted in Arizona over the past four years have yielded new insights into estimating lygus susceptibility to insecticides. Lygus susceptibility was found to vary widely from year to year, from region to region and, for some insecticides, even within the season. It is for this reason that producers need current, region-specific recommendations in order to determine which insecticides are most effective at their locale.

Field efficacy trials are the most direct approach to estimating insecticide efficacy, but for lygus they are expensive to conduct, require large treated and control plots, lygus are notoriously difficult to predict where and when populations will build up, and due to movement of adults, lygus frequently decline in control plots, renderring trial results all but worthless. Laboratory-based bioassays, on the other hand, produce data of uncertain significance. Our studies were intended to improve our understanding of the reliability of glass vial bioassays for estimating efficacy of insecticides used against lygus bugs.

Our results show that the standard glass vial method offers considerable promise for detecting differences in susceptibility of lygus to some, but not all, insecticides. However, mortality in vial bioassays did not serve as a reliable predictor of the relative toxicity of residues of five insecticides in field treatments. Therefore, field evaluations of insecticide efficacy continue to be essential for selecting the insecticides that provide the best control of lygus. Once the most effective materials are selected from field trial results, it is possible to use bioassays reliably and efficiently to monitor changes in population susceptibility to these insecticides. Additional new insights provided by our studies are that efficacy of residues of the insecticides declined rapidly, such that after three days all insecticides caused very little mortality to adult lygus bugs. Lastly, we found a marked dichotomy between residual and direct contact toxicity of the five insecticides evaluated. Even the insecticide treatments that resulted in relatively low toxicity in residual exposure tests killed 95-100% of lygus bugs that they contacted directly under field conditions. This finding indicates that producers experiencing severe problems with lygus control would be well advised to improve insecticide coverage.

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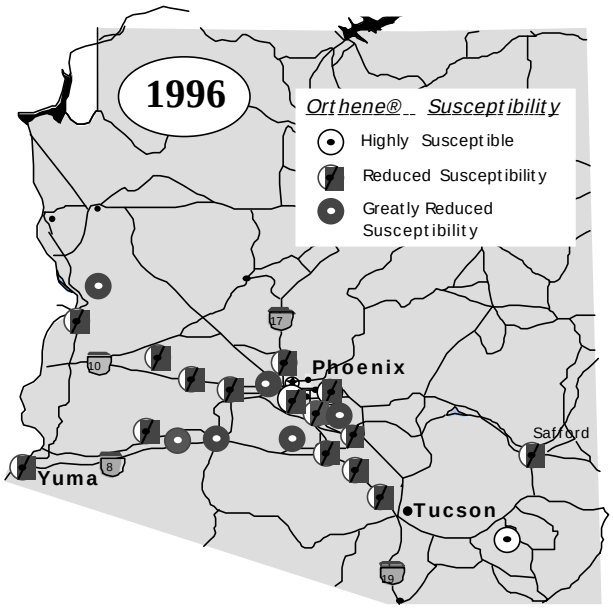

Figure 1. Relative susceptibility to Orthene® of lygus bugs from cotton-producing regions of Arizona in 1996. (From Russell et al. 1997)

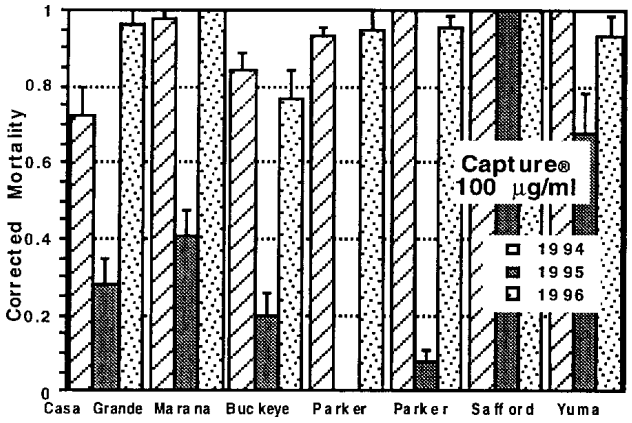

Figure 2a. Changes in Arizona adult lygus bug susceptibility from 1994-1997 as depicted by glass vial bioassay mortality in treatments of 100 µg/ml Capture® (bifenthrin). (From Russell et al. 1997)

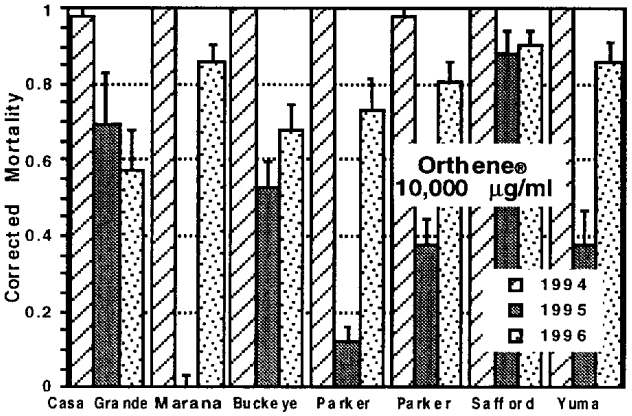

Figure 2b. Changes in Arizona adult lygus bug susceptibility from 1994-1996 as depicted by glass vial bioassay mortality in treatments of 10,000 µg/ml Orthene® (acephate). (From Russell et al. 1997)

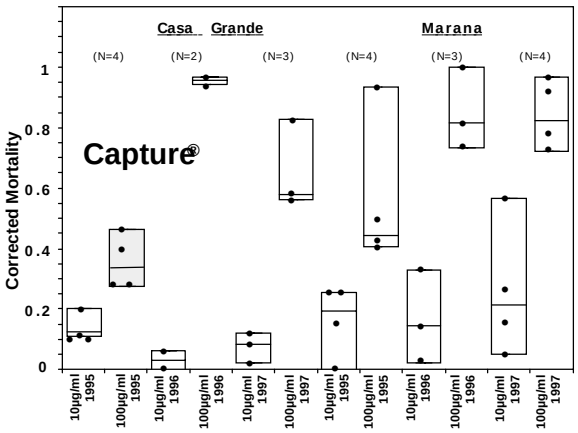

Figure 3a. Within-season variation in mortality observed in glass vial bioassays of 10 and 100 µg a.i./ml Capture® (bifenthrin) of adult lygus bugs tested repeatedly in 1995, 1996, and 1997.

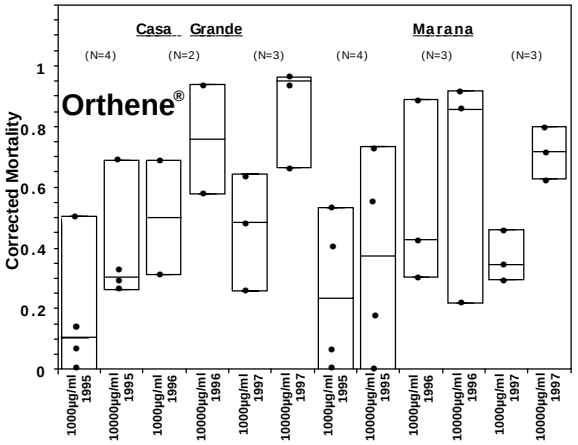

Figure 3b. Within-season variation in mortality observed in glass vial bioassays of 1000 and 10,000 µg a.i./ml Orthene® (acephate) of adult lygus bugs tested repeatedly in 1995, 1996, and 1997.

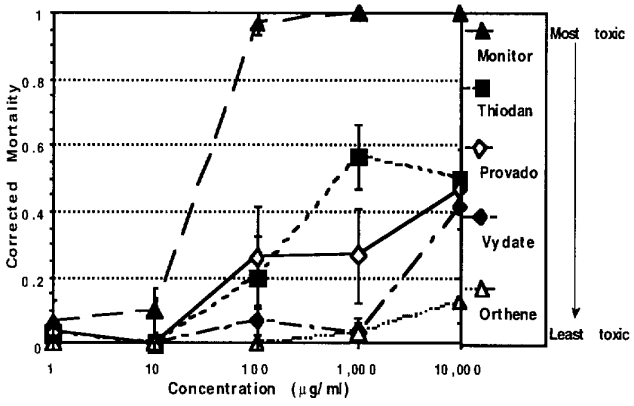

Figure 4a. Estimates from glass vial bioassays of the susceptibility to five insecticides of adult lygus bugs from Central Arizona (Maricopa).

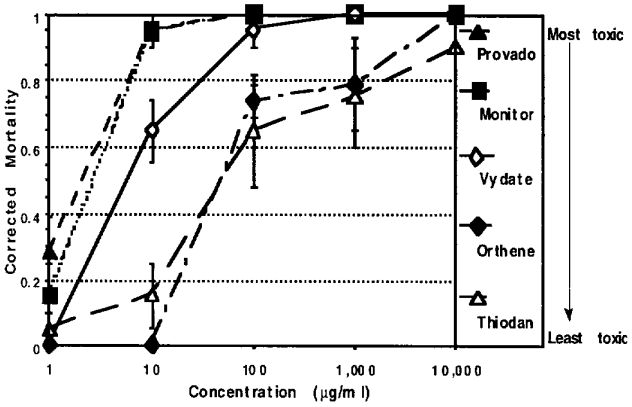

Figure 4b. Estimates from glass vial bioassays of the susceptibility to five insecticides of adult lygus bugs from Eastern Arizona (Safford).

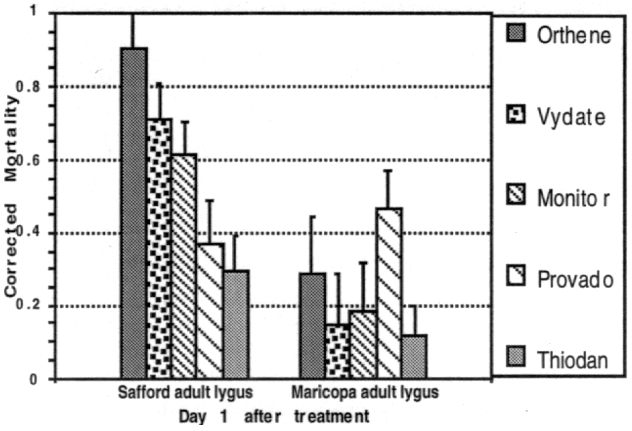

Figure 5b. Susceptibility of adult lygus bugs from Eastern Arizona (Safford) versus Central Arizona (Maricopa) to one-day-old field-weathered residues of five insecticides as depicted by mean mortality observed in cell bioassays.

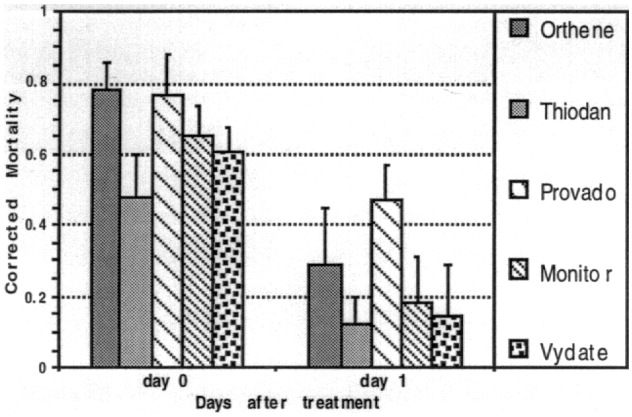

Figure 5a. Susceptibility of adult lygus bugs from Central Arizona (Maricopa) to field-weathered residues of five insecticides as depicted by mean mortality observed in cell bioassays.

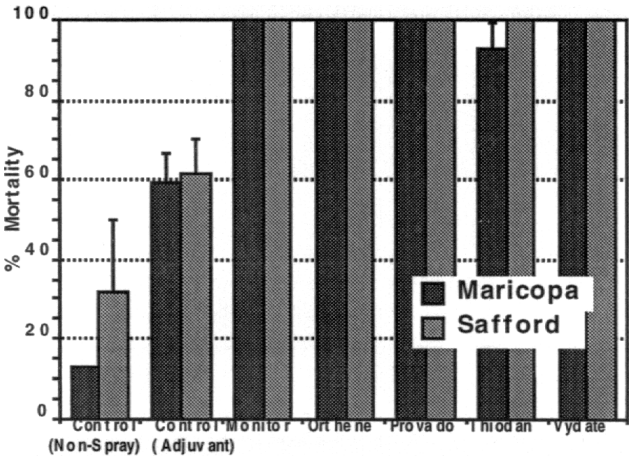

Figure 6. Susceptibility of adult lygus bugs from Central (Maricopa) versus Eastern (Safford) Arizona to direct, topical contact with five insecticides applied under field conditions.