

**MANAGEMENT OF PYRETHROID-RESISTANT
WHITEFLIES IN ARIZONA COTTON:
SELECTION, CROSS-RESISTANCE
AND DYNAMICS**

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Abstract

In 1995, silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, resistance to the widely-used mixture of Danitol® (fenpropathrin) + Orthene® (acephate) was shown to be severe and widespread in Central Arizona cotton. Thereafter, laboratory experiments were undertaken to identify the other major insecticides that were affected by resistance selected by Danitol+Orthene. Large numbers of whiteflies were collected in November of 1995 from the Gila River Basin (GRB) (highly resistant) and the Yuma (relatively susceptible) areas of Arizona. A mixed colony from GRB and Yuma in a 1:4 ratio was established. After six generations of adult selection, appreciable shifts in the concentration responses for pyrethroid, organophosphate, and carbamate insecticides were witnessed, indicating heritable variation for resistance in the source populations. From this we obtained definitive proof that resistance to Danitol+Orthene confers cross-resistance to Asana® (esfenvalerate), Capture® (bifenthrin), Danitol, Decis® (deltamethrin), Decis+Orthene, and Karate® (lambda-cyhalothrin). Additionally, selection with Danitol+Orthene resulted in statistically significant reductions in susceptibility to Curacron® (profenofos), Lannate® (methomyl), Monitor® (methamidophos), and Ovasyn® (amitraz).

Studies were performed to assess tolerance of GRB (pyrethroid resistant) and Yuma (pyrethroid susceptible) populations to a diversity of conventional insecticides currently registered for use in Arizona cotton, with the intention of finding compounds that showed promise for overcoming pyrethroid resistance. Of the materials evaluated, Curacron, Lannate, Lorsban® (chlorpyrifos), Ovasyn, Supracide® (methidathion), and Vydate® (oxamyl) were most promising based on reductions in the two strains of insecticide response differences relative to their widely different responses to Danitol+Orthene.

To determine to what degree pyrethroid resistance in cotton influenced resistance in winter vegetables and melons, and *vice versa*, whitefly populations from spring melons, cotton, and fall melons and cole crops were

collected from Western and Central Arizona regions. In most instances, the whiteflies found in Western Arizona were significantly more susceptible to Danitol+Orthene than those found in Central Arizona. Significant decreases were found in susceptibility to Danitol+Orthene during the 1996 season at three of the four locations in which multiple crops were monitored. This emphasizes that pyrethroid resistance levels can be increased in whitefly populations from any of the cotton, melons, or other winter vegetable crops evaluated. Therefore, management of pyrethroid resistance in Arizona cotton will require harmonizing resistance management efforts and specifically limiting pyrethroid use in the entire crop complex.

Introduction

Silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, is one of the most damaging agricultural insect pests and virus vectors worldwide, attacking vegetables, agronomic and ornamental crops, and weeds (Byrne et al. 1990). It has caused extensive crop damage by direct feeding and through transmission of plant viruses. Outbreaks of this pest have been attributed to the development of insecticide resistance, an outcome of the use of broad spectrum insecticides (Prabhaker et al. 1985, Horowitz et al. 1988, Dittrich et al. 1990, Wool and Greenberg 1990). Reliance on chemicals for whitefly control has resulted in resistance to virtually all classes of registered insecticides and is presently a serious constraint on effective control of this pest in many countries.

Mixtures of pyrethroid+organophosphate combinations have been used extensively in Arizona to control silverleaf whitefly. These combinations have been justified on the premise that mixtures could restore effective control when pyrethroid resistance is solely the result of enhanced esterase activity (Byrne et al. 1994). However, based on studies of Israeli whitefly populations, Byrne et al. concluded that use of the mixtures should, in fact, be avoided because a second, non-esteratic mechanism of pyrethroid resistance existed in field populations, and the use of pyrethroid/organophosphate mixtures would result in the rapid selection of populations resistant to the mixtures.

Monitoring of *B. argentifolii* susceptibility to insecticides in Arizona over the past four years has confirmed the existence of high levels of resistance to pyrethroid insecticides and pyrethroid+organophosphate combinations in some regions (Dennehy et al. 1996; S. S., unpublished). In 1995, whiteflies collected from the Maricopa Agricultural Center (MAC) and the Western Phoenix area were highly resistant to many pyrethroids by the end of the cotton growing season, whereas populations from Yuma were relatively susceptible to these chemicals throughout the season. Laboratory selection of field populations with Danitol®+Orthene® in 1995 yielded very

high levels of resistance to Danitol alone and to the mixture with Orthene after 5-6 generations of selection (S. S., unpublished).

A provisional resistance management strategy was formulated in 1995 and evaluated in a 200 acre field trial (Dennehy et al. 1996). The outcome of this field trial illustrated clearly the seriousness of the pyrethroid resistance problems Arizona growers were encountering. Statewide monitoring of whitefly resistance indicated that cross- and multiple-resistance was rendering ineffective essentially all of the registered pyrethroids and many other insecticides. Field control failures were reported in many Arizona locations during the 1995 field-season (Simmons and Dennehy 1996).

The studies described herein were initiated in response to the whitefly resistance crisis in 1995. We elucidated cross-resistance relationships between Danitol+Orthene and the other insecticides used for controlling whiteflies in Arizona and determined the best approaches for controlling pyrethroid-resistant whiteflies. To offer suggestions to growers regarding alternative registered insecticides for controlling whiteflies highly resistant to pyrethroids, we tested a diversity of registered alternatives against recently-collected populations highly resistant and relatively susceptible to the synergized pyrethroids. Lastly, in an attempt to harmonize regional resistance management efforts in cotton with those in nearby vegetable and melon crops in Arizona, we evaluated resistance dynamics throughout 1996 on a cropping systems basis. It is our hope that this effort will foster cross-commodity cooperation in managing resistance of this pest which annually moves in great numbers within and between Arizona crops.

Research Aims and Methodology

Cross-resistance relationships between Danitol+Orthene and other insecticides

A large pool of silverleaf whiteflies was collected in November 1995 from two locations in Arizona: Gila River Basin (pyrethroid resistant) and Yuma (relatively susceptible to pyrethroids). These two strains were reared under laboratory conditions for two months before a composite strain was established by mixing the Yuma and GRB strains in a 4:1 ratio. Four cages each of selected and non-selected populations were maintained on cotton plants.

Prior to selection of the composite strain with Danitol + Orthene, adult whiteflies from each of the four replicate lines destined to be selected or non-selected were bioassayed for tolerance to the following chemicals using the leaf-disk technique (Rowland et al. 1991, Dennehy et al. 1996): A) pyrethroids and pyrethroid mixtures--Asana® (esfenvalerate), Capture® (bifenthrin), Danitol, Danitol+Orthene, Decis®, Decis+Orthene, and Karate®

(lambda-cyhalothrin); B) organophosphates--Curacron® (profenophos), Lorsban® (chlorpyrifos), Monitor® (methamidaphos), and Pennncap-M® (methylparathion); C) carbamates--Lannate® (methomyl) and Vydate® (oxamyl); D) cyclodiene--Thiodan® (endosulfan); E) chloronicotinyl--Provado® (imidacloprid), F) formamidine--Ovasyn® (amitraz).

Upon completing the initial evaluations of susceptibility to the above-listed chemicals, four lines (i.e., individual cages) of the composite strain were independently selected with Danitol+Orthene (technical grade) for 6 generations. After six generations of selection, the 4 selected and 4 non-selected lines were bioassayed again for susceptibility to the 16 chemicals listed above. Data from the four lines were then bulked and plotted as mean and standard error values for mortality of the selected and non-selected groups to specific concentrations. Statistical significance of the difference in response to specific insecticides of the selected and non-selected lines were determined by ANOVA of mean mortality data transformed with $\arcsin\sqrt{x}$ transformation.

Rearing Conditions

Prior to being used for rearing whiteflies, potted cotton plants were grown in large insect-proof cages inside greenhouses. To rear whiteflies under laboratory conditions, large, cylindrical acetate plastic cages (47 cm diameter, 62 cm height) with insect-proof mesh on top and two sleeves to facilitate operations, were used. Lighting was provided by 75 watt, full-spectrum, fluorescent lights and 40 watt incandescent light fixtures operating on a 12 h photo phase. Five 6 week old insect-free cotton plants were placed in each cage. Measures were taken to minimize the risk of contaminating cultures with other sources of whiteflies. Temperature in the rearing room was maintained at $25 \pm 2^{\circ}\text{C}$.

Selection Procedure

Four lines (cages) of the composite whitefly strain were selected separately with Danitol+Orthene. Additionally, four lines of the composite strain were maintained free of selection to serve as controls. In order to determine the appropriate concentration for selecting each generation, bioassays were conducted on each selected line one week after the onset of adult emergence. These involved coated glass vials and a series of concentrations of Danitol+Orthene. Selection was conducted for 6 generations. Adult whiteflies of the individual lines were selected for resistance by exposing them to treated glass vials at doses sufficient to give 60-80% mortality in 3 h (Sivasupramaniam et al. in press). The survivors were then transferred to new cotton plants held in clean cages for oviposition and development. The minimum number of surviving adults that were used as parents for any generation of a selected strain was >300.

Controlling Pyrethroid-Resistant Whiteflies with Registered Alternatives

Representative pyrethroid-resistant and -susceptible field populations were collected from locations in Maricopa (Gila River Basin) and Yuma (Yuma Agricultural Center) Counties, respectively, in November 1995. They were maintained in cages on cotton plants in the laboratory. Leaf-disk bioassays were then performed to assess susceptibility of the two populations to Danitol+Orthene and to the following non-pyrethroid insecticides: Curacron, Lannate, Lorsban, Ovasyn, Supracide® (methidathion), and Vydate. In addition, we evaluated the newly-registered (in Arizona cotton) pyrethroid Decis®, with and without Orthene.

Pyrethroid Resistance Dynamics in the Cotton, Vegetable, and Melon Cropping Systems in Arizona

Statewide monitoring of resistance showed that silverleaf whiteflies in Central Arizona were generally highly resistant to synergized pyrethroids by the end of the season in 1995. Yet, populations from the Western and some other cotton growing regions in Arizona were relatively susceptible to these chemicals throughout the season (Dennehy et al. 1996). This discrepancy has been attributed to the insecticide-use patterns in the two localities, as well as to the abundance of un-sprayed host plants. We attempted to determine to what degree pyrethroid resistance in winter vegetables and melons influenced the same in cotton, and *vice versa*. Populations from the cotton, vegetable, and melon cropping systems were sampled throughout the 1996 season from Western and Central Arizona regions, and susceptibility to Danitol+Orthene was determined using the leaf-disk bioassay technique.

Results and Discussion

Cross-resistance Relationships Between Danitol+Orthene and other Insecticides

The selected lines showed a dramatic increase in resistance to Danitol+Orthene after 6 generations of selection (Fig. 1a). The non-selected control groups responded very similarly at the beginning and end of the experiment. After six generations, each line selected with Danitol+Orthene achieved levels of resistance in excess of those previously shown to be associated with field-failure of Danitol+Orthene (Simmons and Dennehy 1996).

Cross-resistance relationships were evaluated by recording toxicity of specific insecticides to the composite strain, pre- and post-selection. Resistance to Danitol+Orthene was associated with reductions in susceptibility to all other pyrethroids evaluated: Asana (Fig. 1b), Capture (Fig. 1c), Danitol (Fig. 1d), Decis (Fig. 1e), Decis+Orthene (Fig. 1f), and Karate (Fig. 1g). Therefore, we now have definitive evidence that the resistance of Arizona whiteflies to synergized pyrethroids extends to all the major pyrethroid products currently used for whitefly control in Arizona.

Selection with Danitol+Orthene also resulted in statistically significant reduction in susceptibility to Curacron, Lannate, Monitor, and Ovasyn.

Controlling Pyrethroid-Resistant Whiteflies with Registered Alternatives

At the peak of the whitefly resistance crisis of 1995, Central Arizona cotton producers were trying many different insecticide mixtures to combat whiteflies that had survived repeated treatments of synergized pyrethroids. Many conflicting opinions emerged regarding the merits of various non-pyrethroid materials for overcoming pyrethroid resistance. Evaluations in the laboratory of representative susceptible and resistant field populations confirmed that the insecticides Curacron (Fig. 2a), Lannate (Fig. 2b), Lorsban (Fig. 2c), Ovasyn (Fig. 2d), Supracide (Fig. 2e), and Vydate (Fig 2f) did not appear to be compromised by resistance to Danitol+Orthene as expressed in the GRB whiteflies. This finding does not mean that these insecticides are unaffected by whitefly resistance in Arizona, it merely provides additional evidence that the high levels of resistance to pyrethroids are not linked to reductions in susceptibility to these compounds in the resistant population evaluated. As such, these insecticides may offer utility for controlling pyrethroid resistant whiteflies and for diversifying our insecticide use regime in Arizona cotton.

Pyrethroid Resistance Dynamics in the Cotton, Vegetable, and Melon Cropping System in Arizona

Whitefly populations collected from areas that exhibited resistance buildup in 1995 were consistent in their response to Danitol+Orthene throughout 1996. The high levels of resistance exhibited by populations from Central Arizona underscore the importance of using alternative control methods for combating whiteflies. The differences between locations illustrate why it is not appropriate to provide single recommendations for chemical control of whiteflies statewide. Insecticides that are effective against whiteflies in Western Arizona may not be effective in Central Arizona.

Distinct regional differences were found in whitefly susceptibility to Danitol+Orthene in cotton, melons, and vegetable crops. Significant within-season decreases were found in susceptibility during the 1996 season at three of the four locations in which multiple crops were monitored (Figs. 3a-d). This emphasizes that pyrethroid resistance can be induced in whiteflies in cotton, melons, or other winter vegetables. Therefore, management of pyrethroid resistance in Arizona cotton will require harmonizing resistance management efforts and specifically limiting pyrethroid use in the entire crop complex.

Summary

We have shown that resistance to Danitol+Orthene confers cross-resistance to Asana, Capture, Danitol, Decis,

Decis+Orthene, and Karate. Curacron, Lannate, Lorsban, Ovasyn, Supracide, and Vydate showed promise for controlling pyrethroid-resistant whiteflies in Arizona. Pyrethroid susceptibility levels were very similar in whiteflies from cotton, melons, and vegetables in 1996. Managing resistance by limiting pyrethroid use and capitalizing on the propensity of this resistance to decline will require cross-commodity cooperation to harmonize overall chemical use in regions where cotton is grown in close proximity to vegetables and melons.

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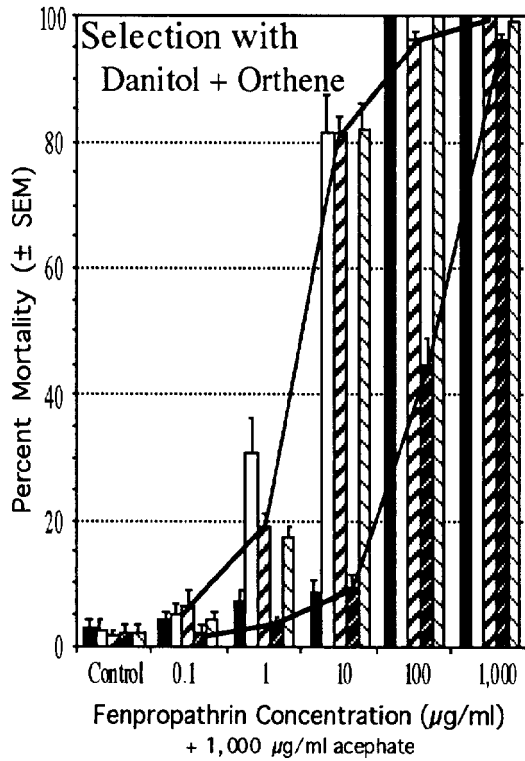
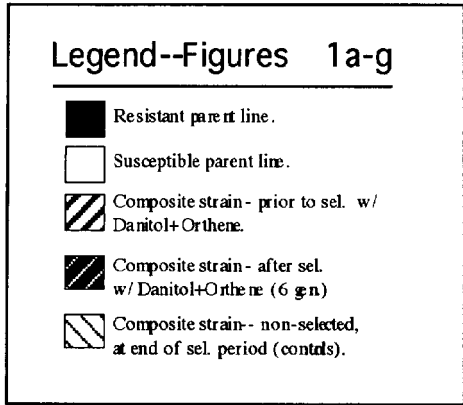


Figure 1a. Susceptibility to mixtures of Danitol® (variable concentrations) and Orthene® (fixed concentration) of a composite whitefly population before and after selection for six generations with the same insecticide mixture. The composite population was established from parental lines collected in Yuma (relatively susceptible) and Maricopa (resistant) Counties, combined in a 4:1 ratio. Responses of the parental lines and unselected groups (controls) are also shown.

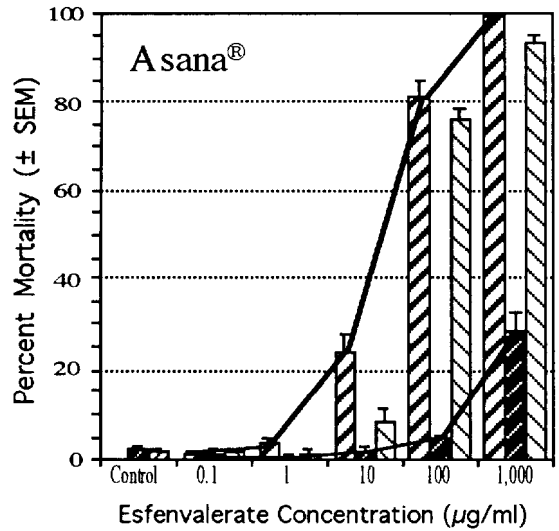


Figure 1b. Selection with Danitol® + Orthene® significantly reduced Arizona whitefly susceptibility to Asana®.

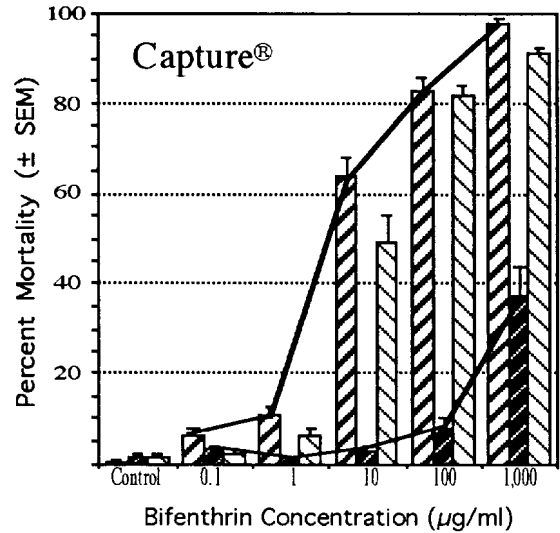


Figure 1c. Selection with Danitol® + Orthene® significantly reduced Arizona whitefly susceptibility to Capture®.

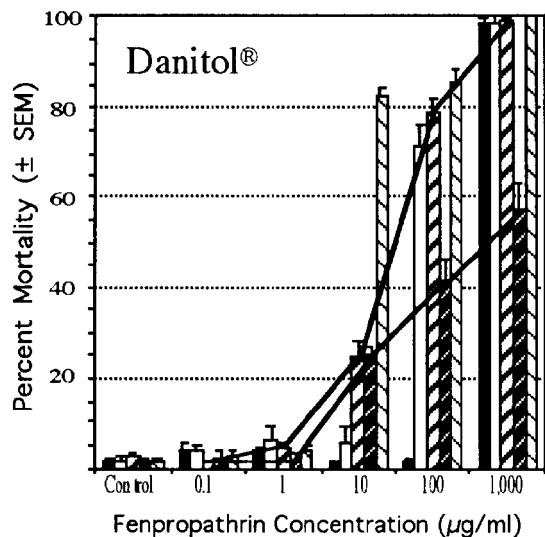


Figure 1d. The reduction in whitefly susceptibility to Danitol® alone was of similar magnitude to that of Danitol® + Orthene® in response to selection with the latter mixture.

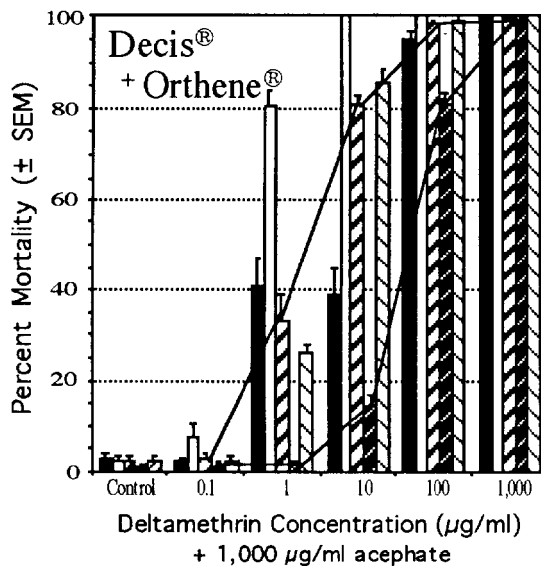


Figure 1f. The reduction in whitefly susceptibility to Decis® + Orthene® was very similar to that of Danitol® + Orthene® in response to selection with the latter mixture.

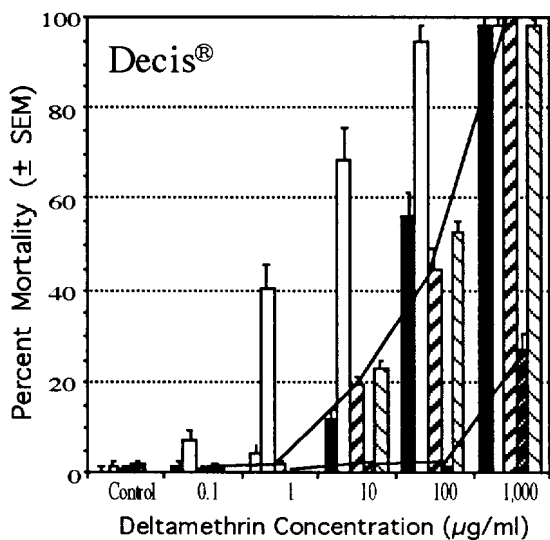


Figure 1e. Selection with Danitol® + Orthene® significantly reduced Arizona whitefly susceptibility to Decis®.

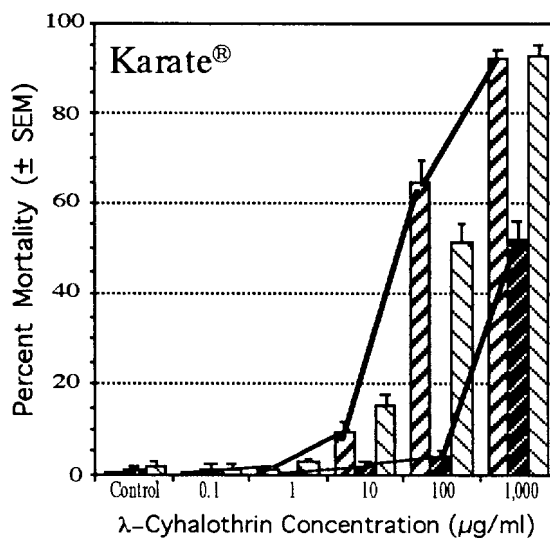


Figure 1g. Selection with Danitol® + Orthene® significantly reduced Arizona whitefly susceptibility to Karate®.

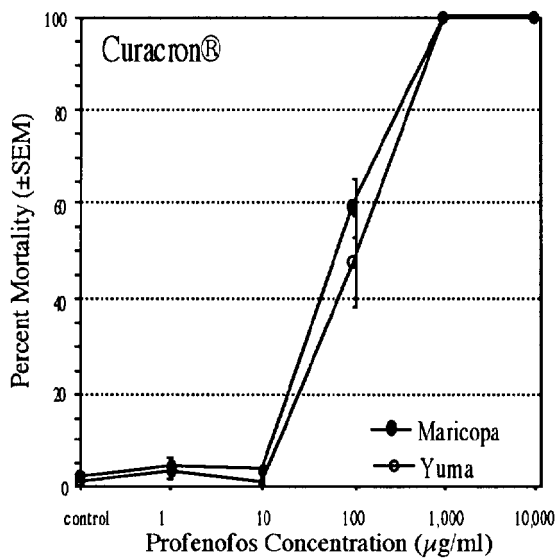


Figure 2a. Toxicity of Curacron® to Arizona whiteflies highly resistant and relatively susceptible to pyrethroid insecticides.

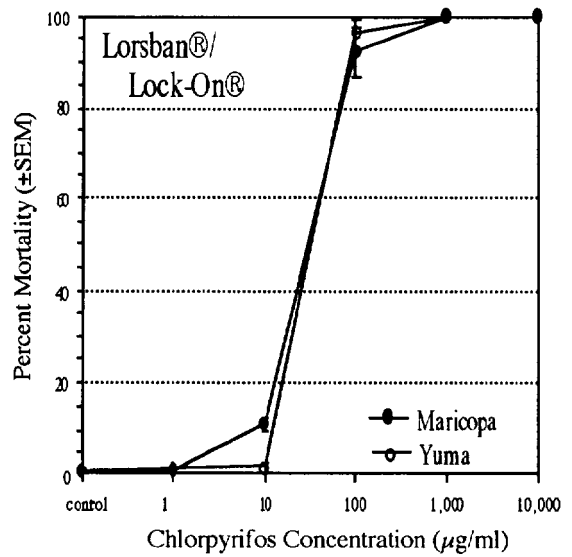


Figure 2c. Toxicity of Lorsban® / Lock-on® to Arizona whiteflies highly resistant and relatively susceptible to pyrethroid insecticides.

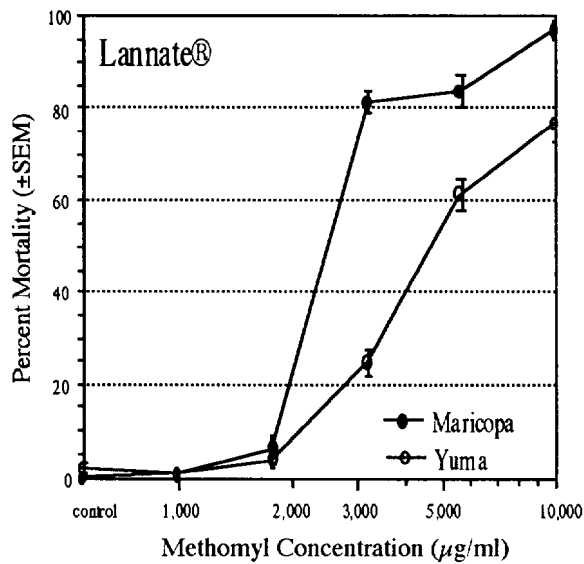


Figure 2b. Toxicity of Lannate® to Arizona whiteflies highly resistant and relatively susceptible to pyrethroid insecticides.

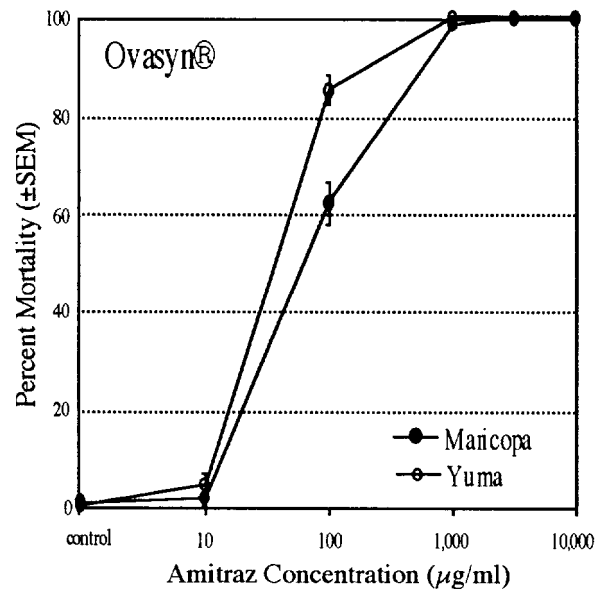


Figure 2d. Toxicity of Ovasyn® to Arizona whiteflies highly resistant and relatively susceptible to pyrethroid insecticides.

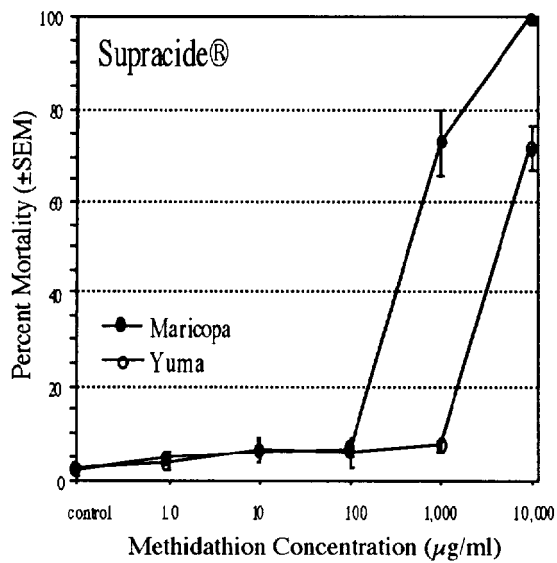


Figure 2e. Toxicity of Supracide® to Arizona whiteflies highly resistant and relatively susceptible to pyrethroid insecticides.

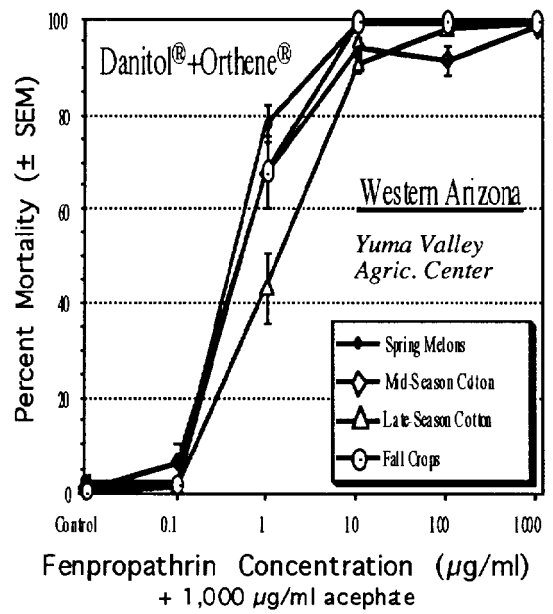


Figure 3a. Changes in susceptibility of pyrethroid insecticides of relatively susceptible whiteflies collected in 1996 from a succession of crops grown at the Yuma Valley Agricultural Center, Yuma Arizona.

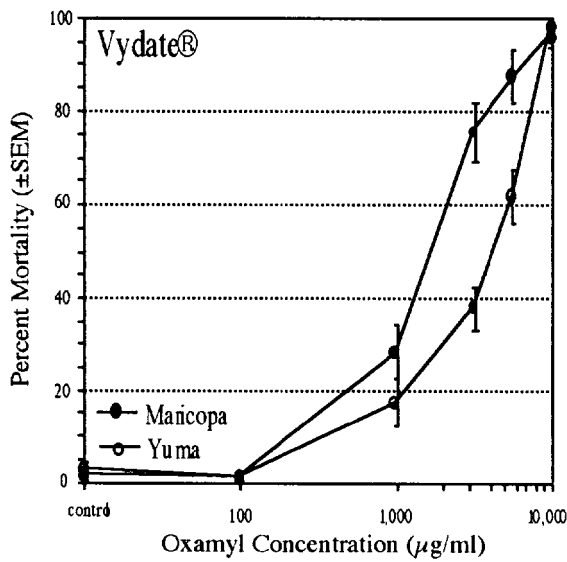


Figure 2f. Toxicity of Vydate® to Arizona whiteflies highly resistant and relatively susceptible to pyrethroid insecticides.

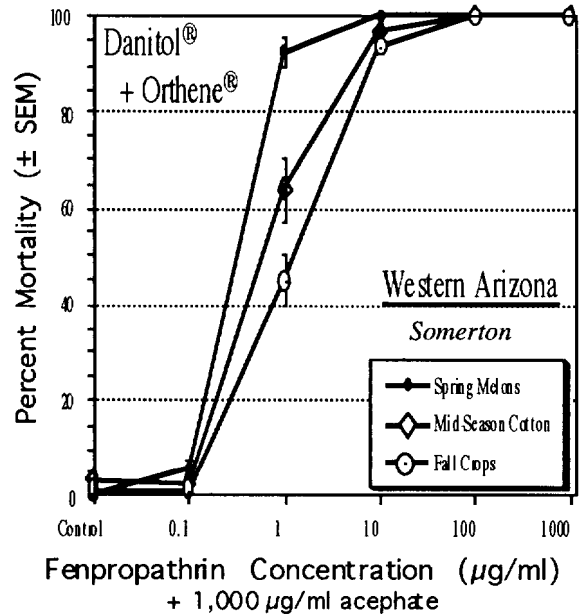


Figure 3b. Changes in susceptibility to pyrethroid insecticides of relatively susceptible whiteflies collected in 1996 from a succession of crops grown near Somerton, Arizona.

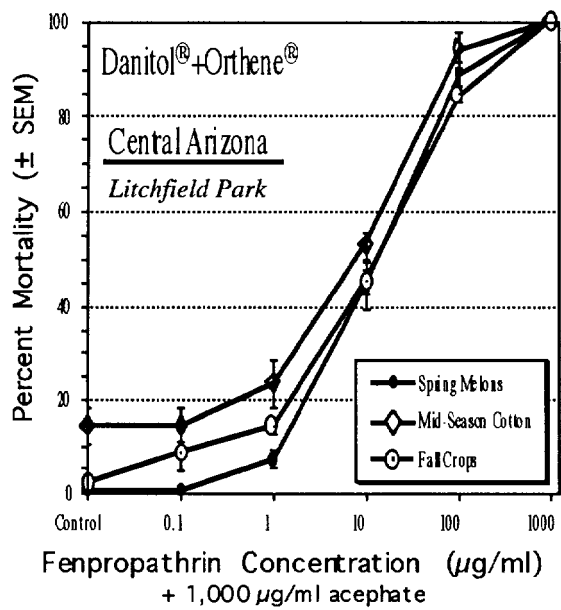


Figure 3c. Changes in susceptibility to pyrethroid insecticides of relatively resistant whiteflies collected in 1996 from a succession of crops grown near Litchfield Park, Arizona.

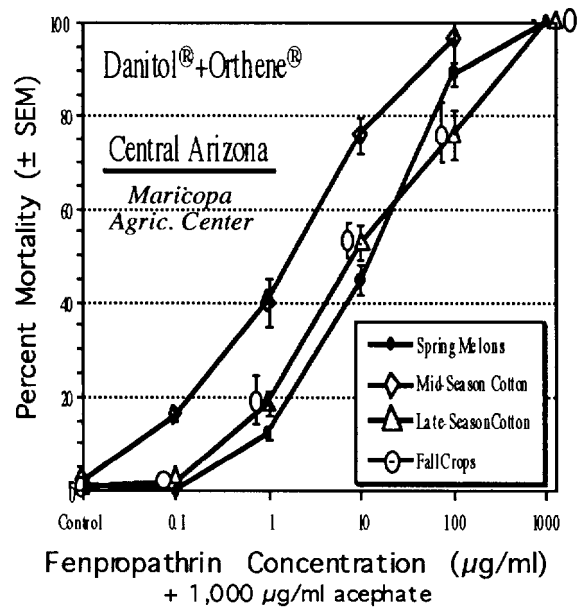


Figure 3d. Changes in susceptibility to pyrethroid insecticides in relatively resistant whiteflies collected in 1996 from a succession of crops grown at the Maricopa Agricultural Center, Maricopa, Arizona.