DEFINING THE RISK OF RESISTANCE TO IMIDACLOPRID IN ARIZONA POPULATIONS OF WHITEFLY

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Abstract

A resistance management program for imidacloprid was initiated in Arizona in 1995, the ultimate goal of which is to sustain the efficacy of this insecticide against *Bemisia*. The current paper reviews our progress toward defining the risk of resistance to imidacloprid in Arizona whiteflies. Bioassay methods for adult whitefly consisted of a 1 day hydroponic uptake by cotton seedlings, followed by a 2 day exposure period. Results from statewide monitoring indicate that whitefly populations throughout Arizona are susceptible to imidacloprid; however, slight increases in resistant whiteflies were observed in 1996, as compared to 1995. Thus far, selection studies with various Arizona whitefly populations have not led to reduced susceptibility to imidacloprid. In a study exploring the influences of different cropping systems on imidacloprid use, we found no major differences in susceptibility to imidacloprid between populations of whiteflies in central and southwestern Arizona. Continued effective management of Arizona whitefly will, in part, hinge on our ability to more effectively integrate our knowledge of whitefly biology with resistance management strategies.

Introduction

The silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, is an important pest of cotton, vegetables, and melons in southern Arizona (Byrne et al., 1990; Palumbo 1994a and b). Imidacloprid, an insecticide of the chloronicotinyl group that exhibits both systemic and contact activity, plays an important role in whitefly suppression on many crops. The systemic formulation (Admire®) serves a critical role in whitefly control in vegetables and melons, due to its long residual activity (Mullins 1993; Palumbo 1994b; Palumbo et al., 1994; Palumbo 1995). The extreme effectiveness of Admire® in vegetables and melons has meant that fewer whiteflies move into cotton in the spring. Therefore, efficacy of Admire® in vegetables is very much of concern to cotton growers. The foliar formulation of imidacloprid (Provado®) is used in cotton to control whiteflies, as well as plant bugs and aphids (Mullins and Christie 1995). Also, other chloronicotinyl compounds are slated for registration in cotton in the near future. The extensive use of chloronicotinyl compounds on a broad range of whitefly hosts, coupled with the whitefly's capacity to disperse between crops, puts this entire class of compounds at great risk for the development of resistance. Importantly, for Arizona cotton growers to sustain their newly-achieved success in whitefly management (see Dennehy et al., this volume), it will be necessary to manage resistance problems in vegetables and melons, as well as cotton.

Recent studies have documented whitefly resistance to imidacloprid. In California, researchers found 50-fold resistance to imidacloprid was selected relatively rapidly in whitefly populations from the Imperial Valley (Prabhaker et al., 1995). However, to date there has been no report of field strains possessing this resistance in the Imperial Valley and the impact on field performance of imidacloprid of the 50-fold resistance created in the laboratory remains unknown. Widespread resistance to imidacloprid, resulting in reports of field failures, has been reported from the Almeria region of Spain after only two years of use (Cahill et al., 1996). In many respects, agricultural production in Almeria is similar to that in southwestern Arizona. In both areas, melons and vegetables are produced year around and are high value crops with relatively low pest tolerances. Furthermore, mild weather and continuous cropping sustain at least moderate whitefly densities throughout the year, and in response to this threat, applications of imidacloprid are routine.

The similarities between Spain and Arizona should serve as a warning to Arizona vegetable and cotton growers that Arizona whitefly are likely to soon develop resistance to chloronicotinyl compounds. Given the pivotal role this group serves in whitefly control it is only reasonable that we be prepared for the inevitable development of resistance and begin now to ask critical questions about how our growers should respond to this situation in the future. Therefore, in 1995 we initiated a resistance management program for imidacloprid, the ultimate goal of which is to sustain the efficacy of this insecticide against *Bemisia*. This paper presents our progress in defining the risk of resistance to imidacloprid in Arizona populations of whiteflies.

Development of Bioassay Methods

Efficient and reliable bioassay methods are a prerequisite for maintaining an effective resistance management program. Because our first concern with imidacloprid is in vegetables and melons, where it is used as a soil treatment, we developed a systemic-uptake bioassay that exposed whitefly adults to the chemical through feeding on the leaf (Williams et al., 1996). The following is a brief summary

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of the method including a minor modification made to improve efficiency. For further details see Williams et al. (1996). Cotton seedlings, *Gossypium hirsutum* L. (var. DPL-50), at the second true leaf stage were excised at the mainstem and placed in the desired concentration of imidacloprid for 1 day of hydroponic uptake. Leaf disks (2.5 cm diam) were excised from the true leaves and placed in 20 ml glass vials over a thin layer of agar gel. Twenty-five adult whiteflies were aspirated into each vial, after which vials were capped with dialysis membrane. After a 2 day exposure period, mortality was assessed by observing individuals in each vial with a dissecting scope. Individuals not exhibiting repetitive (non-reflex) movement were scored as dead.

Baseline Susceptibility of Arizona Populations

In order to be in a position to detect resistance development to imidacloprid as early as possible it is necessary to have a broad knowledge of the baseline susceptibility to imidacloprid of Arizona populations. Thereafter, routine monitoring for changes in susceptibility is essential for disclosing and responding to resistances before they become widespread and cause losses to growers due to ineffective insecticide applications. It is our objective to provide growers with real-time information on the imidacloprid resistance status of whitefly populations throughout Arizona. A Yuma population was placed in culture in 1993, and evaluated for susceptibility to imidacloprid prior to any exposure to imidacloprid. In 1995, whiteflies were evaluated from eight locations throughout the cotton-growing regions of Arizona (Williams et al., 1996). We expanded our efforts in 1996 to include 18 locations, seven of which were part of the 1995 study. At each location in 1996, approximately 8000 whiteflies were collected in plastic vials (13 dram) by vacuuming foliage with a Makita Cordless Vacuum® (Model 4071D). Samples were chilled and transported to the Extension Arthropod Resistance Management Laboratory, in Tucson, within 8 hours. At the Laboratory, samples were released into cages containing several cotton, *G. hirsutum* L. (var. DPL-50), plants at the 5-7 true leaf stage. Adults were bioassayed as described above, approximately 36 hours after field collection.

Many Arizona populations never exposed to imidacloprid exhibit a plateau in concentration-response to imidacloprid (Fig. 1). We have examined this phenomenon extensively and it persists with both foliar and systemic bioassay methods and under a variety of different conditions. The plateau suggests the presence in Arizona whitefly populations of genes conferring reduced susceptibility to imidacloprid. This finding prompted us to initiate the studies, described herein, to select for imidacloprid resistance. Also, we then conducted statewide evaluations of whitefly susceptibility to imidacloprid using concentrations spaced on log intervals ranging from 0.1 to 1000 µg/ml imidacloprid. We have found little geographic variation in susceptibilty of Arizona populations to imidacloprid (Tables 1 and 2). In 1995, whitefly populations from four of eight locations had survivors in 1000 µg/ml bioassays (Table 1). In 1996, survivors at this concentration were observed in 14 of 18 populations (Table 2). At the seven sites which were studied both years, there was a statistically significant (P=0.05) increase in the proportion of survivors of 1000 µg/ml in 1996. Although survivors of this high concentration of imidacloprid comprise only a small proportion of whiteflies tested, 0.6% in 1995 and 5% in 1996, our findings suggest that the proportion of highly resistant individuals is increasing slowly in Arizona.

Isolation and Selection for Resistant Strains

The striking plateau in response of Arizona whitefly populations to imidacloprid and the related finding of increasing proportions of whiteflies surviving high concentrations in bioassays led us to conclude that we should be able to rapidly select for a high level of imidacloprid resistance in the laboratory. This has not been the case. Thus far, selection with imidacloprid of various Arizona populations has not increased resistance (Fig. 2). This finding is somewhat of an enigma, since our data suggest that resistance levels are creeping upward in field populations. At least for the present time, however, imidacloprid continues to provide control of Arizona whitefly. Once highly resistant populations are isolated, future work will focus on the stability of imidacloprid resistance, and characterization of cross-resistance relationships.

Resistance in the Arizona Cotton-Vegetable-Melon Ecosystem

Arizona cotton growers achieved a major success in whitefly control in 1996 with the introduction of two new insect growth regulators and an integrated resistance management program. However, this could be a short-lived success if resistance problems flare in desert vegetable or melon crops. Of greatest concern in this regard is the fact that imidacloprid assumes such great importance for whitefly control in these crops. Simply put, loss of efficacy of imidacloprid in vegetables and melons would likely wreak havoc on cotton in the vicinity and could cause us to revisit the circumstances that brought about the emergency registrations of imidacloprid in 1993 in Arizona. It is for this reason that we are devoting attention to the detection and management of resistance in the cotton-vegetable-melon ecosystems of central and western Arizona.

Differences in Cropping Systems

Vegetables, melons, and untreated whitefly (or seldom treated) hosts, such as alfalfa, are much more abundant in some cotton growing areas of Arizona than others. We wish to determine the affect of such differences in crop types and patterns on the development of imidacloprid resistance. For example, in 1995 there was an estimated 224,500 acres of whitefly hosts in Maricopa County (central Arizona), and 122,450 acres in Yuma County (southwestern Arizona) (Fig. 3). Cotton comprised nearly two-thirds of the cultivated whitefly host plants in Maricopa County, but less than one-quarter of them in Yuma County. This large difference in the proportional significance of cotton may explain why whitefly resistance to the synergized pyrethroids was insignificant in western Arizona while at the same time reached crisis levels in central Arizona (Dennehy et al., 1996).

Vegetables accounted for more than half of the cultivated whitefly hosts in Yuma County, but only about 10% in Maricopa County in 1995. Alfalfa comprises an important reservoir crop for whiteflies and is seldom treated with insecticides that kill whiteflies. In both Maricopa and Yuma Counties alfalfa made up about one-quarter of the available hosts for whiteflies.

Differences in Imidacloprid Use

Data on imidacloprid use in 1995 were obtained from a combination of L1080 documents collected by the Arizona Department of Agriculture and survey data collected by the Arizona Agricultural Statistics Service (W. Sherman, pers. comm.). Seven times more imidacloprid was applied in Yuma County than in Maricopa County (5895 vs. 823 lbs. a.i.). Yuma County received 13 times more imidacloprid per acre than did Maricopa County (Fig. 4), and application rates were highest on Yuma County vegetables.

Differences in cropping systems also influenced the formulation of imidacloprid applied. In Yuma County, 99% of the imidacloprid was applied to the soil as a systemic (Admire®) in vegetable and melon production (Fig. 5). However, in Maricopa County, 90% was applied as a foliar spray (Provado®) on cotton. Due to the chemical properties of imidacloprid, its dissipation rate is greatly affected by application conditions. Imidacloprid residues remain in the soil for upwards of 100 days (Scholz and Spiteller 1992; Mullins 1993). However, when mixed with water and exposed to sunlight, imidacloprid dissipates after only a few days (Mullins 1993).

Whitefly susceptibility to imidacloprid is being monitored throughout the year at sites at which cotton, vegetables, melons, and alfalfa are grown. Figure 6 presents results from two sites that typify the trends observed thus far. At the Maricopa Agricultural Center (central Arizona) whitefly mortality in bioassays of 1000 µg/ml ranged from 80-100%. At Dome Valley (southwestern Arizona) mortality at 1000 µg/ml ranged from 85-100%. Thus, we have not observed major shifts in susceptibility to imidacloprid between whitefly populations in central and southwestern Arizona.

The implications of these data for the development of imidacloprid resistance are clear. Due to the abundance of vegetables, whiteflies in Yuma County are subjected to much more exposure to imidacloprid than in Maricopa County. Moreover, vegetable production intensifies resistance selection because of widespread use of the systemic formulation of imidacloprid. Therefore, when resistance begins to impair field performance of imidacloprid in Arizona, we expect to see it first in western Arizona. As noted above, reductions measured to date have been small but the proportion of resistant individuals appears to be increasing. We will continue our cropping systems investigations of whitefly resistance to imidacloprid in the cotton-producing regions of the State, and intensify work in southwestern Arizona.

Summary

- Management of resistance to chloronicotinyl whitefly materials is of great importance to Arizona cotton, vegetable, and melon producers.
- We observed slight but statistically significant increases in highly resistant whiteflies between 1995 and 1996.
- Statewide monitoring indicate that populations remain susceptible throughout Arizona.
- We have seen no signs of field failure of imidacloprid in Arizona.
- There were no major differences in susceptibility of whitefly populations from melons, cotton, or vegetables in Maricopa and Yuma Counties.
- Despite clear indications of the presence of resistance genes in unexposed Arizona populations, selection of whiteflies in the laboratory did not appreciably reduce susceptibility to imidacloprid.
- There is an urgent need to harmonize chemical use and resistance management efforts in cotton, vegetables, and melons in Arizona to avoid conflicts resulting from movement between crops of resistant pests.
- Management of resistance to chloronicotinyl compounds will necessitate limited use across crops which are whitefly hosts, and cross-commodity collaboration and cooperation between growers and researchers.

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Hannan (Barkley Co. of Arizona), and Scott Tollefson (Del Monte Fresh Fruit, Inc.).

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Table 1. Mortality (± std. dev.) of Arizona whitefly populations in systemic leaf disk bioassays with imidacloprid conducted in 1995.

	Concentration Imidacloprid (µg/ml)				
Site	**0**	**1**	**10**	**100**	**1000**
Buckeye	3.70 (3.5)	75.6 (10)	86.6 (7.6)	97.1 (3.8)	98.8 (1.9)
Casa Grande	3.40 (2.7)	40.4 (22)	89.6 (6.9)	92.9 (6.3)	98.4 (2.7)
Dome Valley	5.40 (8.3)	85.5 (5.9)	96.5 (4.0)	98.6 (2.1)	98.6 (2.1)
Gila River Basin #1	10.6 (7.8)	73.9 (14)	85.5 (7.6)	95.3 (6.7)	100 (0)
Gila River Basin #2	9.70 (10)	77.0 (8.8)	93.5 (6.7)	99.2 (2.5)	100 (0)
Gila Valley	8.60 (5.6)	88.1 (8.0)	94.1 (3.6)	98.8 (2.6)	96.5 (3.6)
Maricopa Ag. Ctr.	1.20 (2.7)	61.3 (20)	98.0 (2.9)	99.1 (1.9)	100 (0)
Yuma Valley Ag. Ctr.	15.1 (8.2)	97.3 (3.5)	100 (0)	100 (0)	100 (0)

Table 2. Mortality (± std. dev.) of Arizona whitefly populations in systemic leaf disk bioassays with imidacloprid conducted in 1996.

	Concentration Imidacloprid (µg/ml)					
Site	0	0.1	1	10	100	1000
Buckeye	7.10 (3.5)	35.6 (20)	84.9 (13)	95.9 (4.5)	98.1 (4.5)	95.9 (4.5)
Casa Grande	16.2 (12)	32.0 (15)	76.8 (9.2)	91.8 (6.0)	97.9 (2.2)	97.1 (3.9)
Coolidge	4.30 (4.2)	44.8 (13)	95.7 (3.5)	99.6 (1.2)	100 (0)	100 (0)
Dome Valley	9.60 (9.4)	26.8 (15)	94.8 (6.3)	99.5 (1.5)	100 (0)	100 (0)
Gila River Basin #1	22.6 (14)	44.3 (20)	79.1 (14)	88.1 (10)	92.8 (3.9)	93.5 (3.7)
Gila River Basin #2	11.3 (14)	41.3 (20)	62.4 (24)	83.1 (17)	93.4 (7.6)	96.1 (4.5)
Laveen	2.50 (2.9)	81.1 (10)	95.5 (5.3)	99.6 (1.2)	100 (0)	99.6 (1.4)
Litchfield Park	6.10 (6.0)	94.1 (6.2)	94.1 (5.1)	99.2 (1.6)	100 (0)	100 (0)
Marana	7.10 (7.3)	48.6 (26)	88.1 (5.6)	93.9 (4.4)	97.1 (2.8)	97.7 (4.8)
Maricopa Ag. Ctr.	13.8 (12)	41.2 (13)	79.7 (9.6)	89.8 (10)	95.7 (4.4)	91.7 (5.1)
Mohave Valley	6.10 (11)	20.8 (14)	61.6 (11)	68.1 (13)	86.8 (9.6)	85.6 (9.4)
Paloma	7.70 (9.2)	56.8 (15)	89.1 (4.0)	90.9 (4.5)	100 (0)	96.4 (5.2)
Parker	13.8 (8.1)	79.5 (13)	91.2 (6.7)	92.1 (5.4)	95.3 (5.3)	98.3 (2.2)
Peters Corner	6.20 (4.2)	75.7 (10)	90.5 (6.9)	98.0 (2.1)	100 (0)	98.9 (2.5)
Roll	9.70 (6.2)	16.0 (9.6)	63.7 (13)	91.2 (6.3)	96.2 (3.2)	98.4 (2.1)
Somerton	4.20 (3.3)	42.4 (11)	87.7 (8.7)	93.9 (4.8)	97.1 (5.2)	100 (0)
South Harquahal a Valley	12.1 (11)	94.1 (3.4)	97.4 (3.9)	98.0 (2.1)	100 (0)	98.3 (2.9)
Yuma Valley Ag. Ctr.	5.90 (5.7)	31.9 (17)	76.4 (7.1)	84.6 (6.9)	91.3 (5.1)	90.4 (6.2)

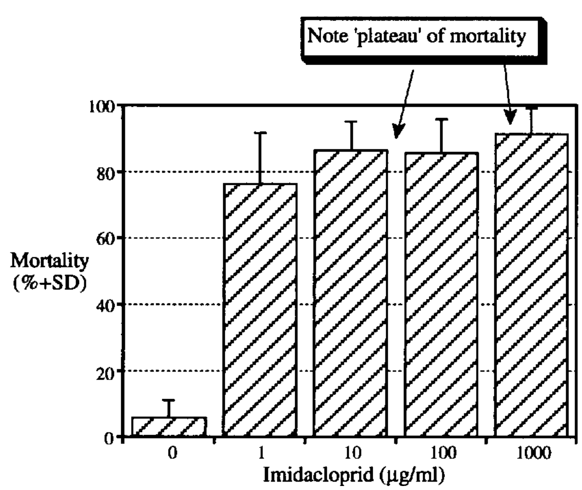

Figure 1. Baseline susceptibility of an Arizona whitefly population never exposed to imidacloprid. Plateau suggests the presence of genes conferring reduced susceptibility to imidacloprid.

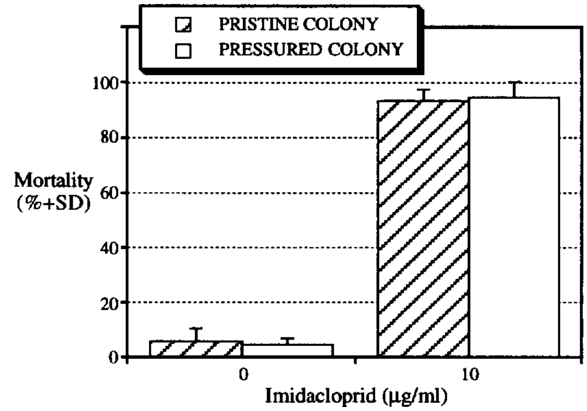

Figure 2. Susceptibility of a pristine whitefly colony (never exposed to imidacloprid) contrasted with that of the same colony continuously exposed to imidacloprid for about 25 generations.

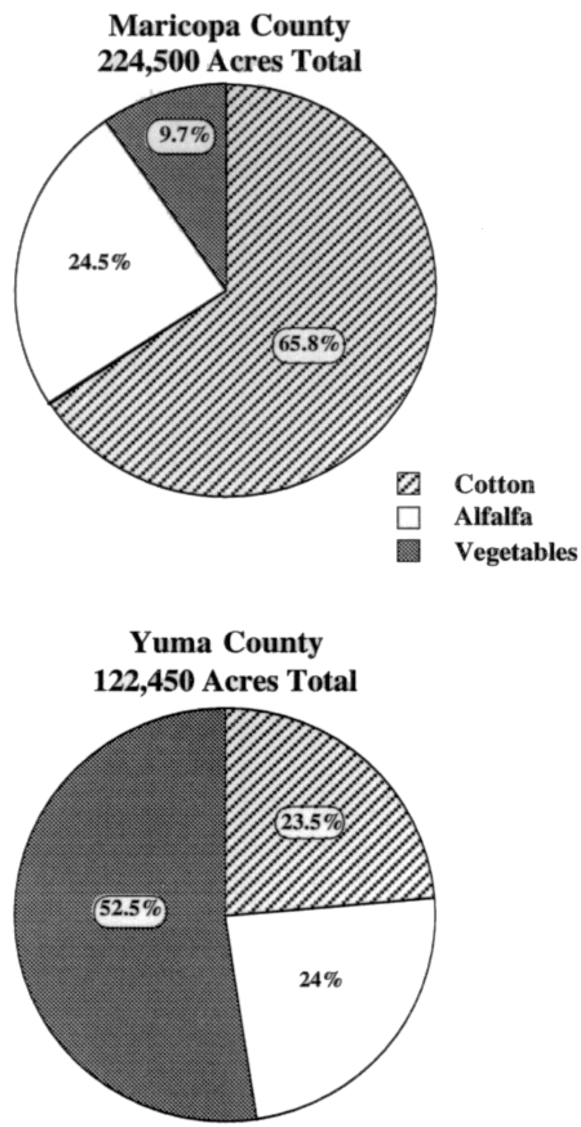

Figure 3. Differences in the acreage of cultivated whitefly hosts in Maricopa County (central Arizona) and Yuma County (southwestern Arizona) in 1995.

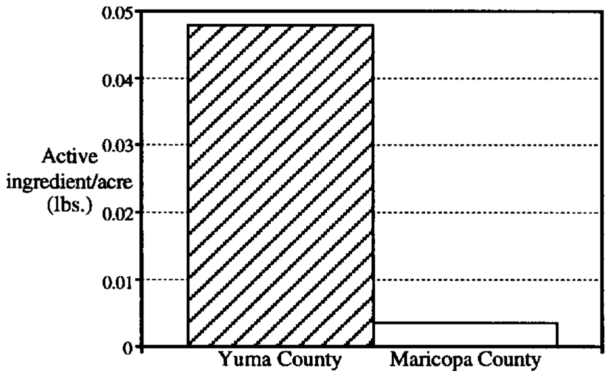

Figure 4. In 1995, an average of 13 times more imidacloprid was applied per acre of whitefly host in Yuma County (southwestern Arizona) than Maricopa County (central Arizona).

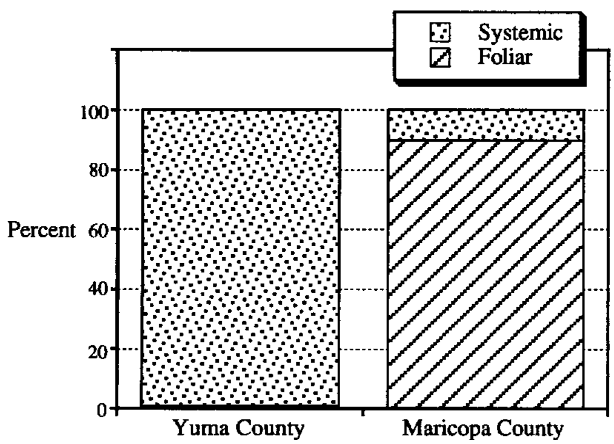

Figure 5. The systemic formulation of imidacloprid (Admire®) comprised 99% of the active ingredient in Yuma County. In Maricopa County, 90% of the imidacloprid was applied in the foliar formulation (Provado®).

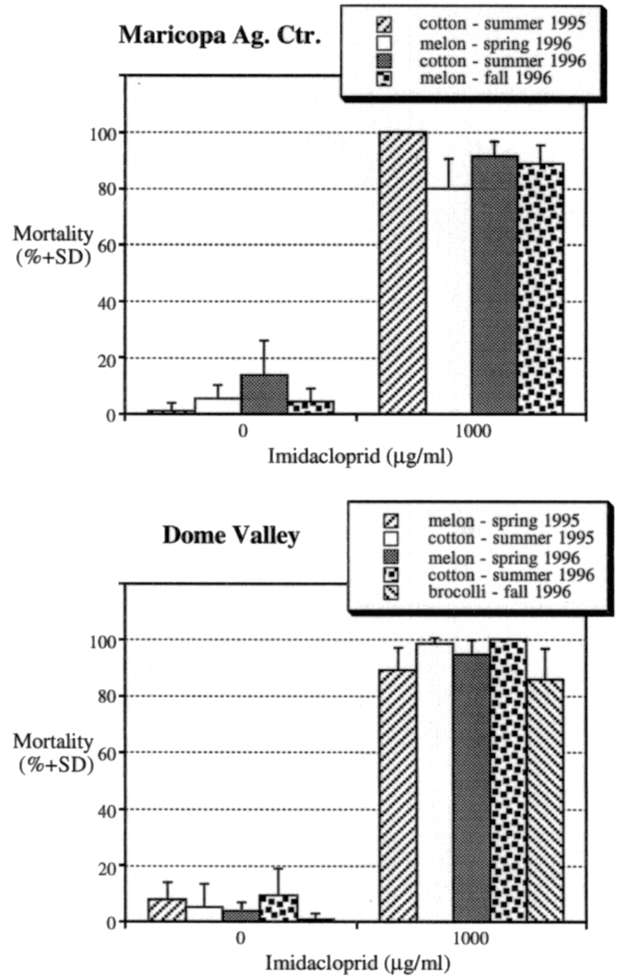

Figure 6. Contrasts of susceptibility to imidacloprid of whitefly populations from the Maricopa Agricultural Center (central Arizona) and Dome Valley (southwestern Arizona) in 1995 and 1996.