CAN RESISTANCE TO CHLORONICOTINYL INSECTICIDES BE AVERTED IN ARIZONA FIELD CROPS?

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Abstract

A resistance management program was initiated in Arizona in 1995, the initial goal of which was to sustain the efficacy of imidacloprid (Admire®) against *Bemisia* in vegetable crops. Due to the anticipated registration of additional chloronicotinyl (and related neonicotinyl) insecticides in Arizona, project objectives were subsequently broadened to address management of this entire class of insecticides in Arizona field crops. Results from three years of statewide monitoring of whiteflies from cotton indicated that whitefly populations in Arizona have become significantly less susceptible to imidacloprid in each of the past two years and significant geographical differences were described. However, no evidence was found of reduced field performance of imidacloprid in vegetables. Additionally, laboratory studies subjecting Arizona whiteflies to selection with imidacloprid did not increase levels of resistance beyond those occurring in the field. A study exploring the influences of cropping systems differences on imidacloprid use (Admire® and Provado®) revealed no major differences in susceptibility to this insecticide between populations of whiteflies in central and southwestern Arizona. However, distinct seasonal shifts to lower susceptibility from 1996 to 1997 were observed in the Dome Valley of Southwestern Arizona. Susceptibility of Arizona whitefly populations to imidacloprid was highly correlated with susceptibility to acetamiprid but was unrelated to susceptibility to CGA-293343. There is an urgent need to harmonize chemical use and resistance management efforts in Arizona cotton, vegetables and melons to avoid conflicts resulting from movement of pests between crops.

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, b). Suppression of *Bemisia* has hinged primarily on insecticides. Imidacloprid, an insecticide of the chloronicotinyl group that exhibits both systemic and contact activity, has served an important role in suppression of Arizona whitefly since its registration in 1993. The systemic formulation (Admire®) provides exceptional control of whiteflies in vegetables and melons, due to its long residual activity (Mullins 1993; Palumbo 1994b; Palumbo et al., 1994; Palumbo 1995). The extreme effectiveness of imidacloprid in vegetables and melons has meant that fewer whiteflies move into cotton in the spring. For this reason, efficacy of imidacloprid in vegetables is very much of concern to cotton growers in Arizona. Intense use on a broad range of whitefly hosts, coupled with the whiteflyÕs rapid life cycle and capacity to disperse between crops, puts imidacloprid at considerable risk for the development of resistance. Moreover, we anticipate registration in Arizona of other chloronicotinyl insecticides in the near future for control of whiteflies and/or other pests.

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 less than three 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 result in routine use of imidacloprid.

Analysis of regional insecticide use patterns in Arizona heighten concerns that *Bemisia* in southwestern Arizona is at great risk for the development of resistance to imidacloprid. Imidacloprid use is intense on fall melons and winter vegetables (principally lettuce and cole crops) grown extensively throughout southwestern Arizona. Generally, when chemical control of whitefly is necessary on other crop hosts, such as cotton, spring melons, and alfalfa, alternative insecticides are used. Thus, there is a period of several months in the spring and summer during which selection for resistance to imidacloprid, even in southwestern Arizona, is relatively low. This temporal discontinuity between crops on which imidacloprid is used is likely an important factor limiting the development of resistance in Arizona.

For Arizona cotton growers to sustain their newly-achieved success in whitefly management (Dennehy and Williams

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1997), it will be necessary to harmonize chemical use in vegetables and melons, as well as cotton. The similarities between the Almeria region of Spain and southwestern Arizona, coupled with the development of severe resistance to imidacloprid in Spain, should serve as a warning to Arizona growers of the consequences of unbridled use of this class of insecticides.

Given the pivotal role this insecticide group serves in Arizona, 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 it in the future. This paper presents an update on progress in that regard.

Development of Bioassay Methods

Efficient and reliable bioassay methods are a prerequisite for routine monitoring of resistance on a statewide basis. Because our first concern with imidacloprid was in vegetables and melons, where it was used as a soil treatment, we modified a systemic-uptake bioassay previously described by Cahill et al. (1996) that exposed whitefly adults to the chemical through feeding on the leaf (Williams et al., 1996). The following is a brief summary of the method. Cotton seedlings, *Gossypium hirsutum* L. (var. DPL-50), at the second true-leaf stage of growth were cut at the mainstem soil line and placed in desired concentrations of imidacloprid for one day of hydroponic uptake. Leaf disks (2.5 cm diameter) were then 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 two-day exposure period, mortality was assessed by observing individuals in each vial with a dissecting scope. Individuals unable to move appendages repetitively (non-reflex) were scored as dead.

Baseline Response of Arizona Whiteflies

Detection of resistance to imidacloprid in its early stages of development requires that a substantial amount of data be collected to document variation within and between unexposed populations. Thereafter, routine monitoring for changes in susceptibility is essential for disclosing and responding to resistance before it becomes widespread and causes economic losses to growers. It has been our objective to provide Arizona growers with real-time information on the imidacloprid resistance status of whitefly populations throughout Arizona.

We have inferred baseline responses of populations in two ways, through contrasts with a pristine population that had never been exposed to imidacloprid, and through contrasts with responses of field populations in areas where imidacloprid use was negligible. A Yuma population from melons was placed in culture in 1993, and evaluated in 1994 for susceptibility to imidacloprid, having never been exposed to imidacloprid. 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 low frequencies of genes conferring reduced susceptibility to imidacloprid. This finding prompted us to initiate the studies, described herein, to select for imidacloprid resistance.

Susceptibility of Whitefly Populations from Cotton

Since 1995 we have monitored on a statewide basis susceptibility to imidacloprid of whiteflies collected from cotton. This effort has allowed us to detect with confidence changes in whitefly susceptibility to imidacloprid. Collections were made each year from June through October at 8, 18, and 14 locations in 1995, 1996 and 1997, respectively.

For each collection approximately 8000 whiteflies were collected in plastic vials (13 dram) by vacuuming cotton foliage with a Makita Cordless Vacuum® (Model 4071D). Samples were chilled and transported to the Extension Arthropod Resistance Management Laboratory, in Tucson, within eight hours. At the Laboratory, samples were released into cages containing several cotton, *G. hirsutum* L. (var. DPL-50), plants at the five to seven true-leaf stage. Adults were bioassayed approximately 36 hours after field collection. Bioassay concentrations ranged from 0.1 to 1000 µg/ml imidacloprid.

Results of 1997 monitoring are presented in Table 1; results of 1995 and 1996 monitoring were reported previously (Williams et al. 1996, 1997). In each of the past two years we have detected small but significant overall reductions in statewide means of cotton whitefly survivorship to Imidacloprid (Fig. 2). Mean survivorship of all populations tested in 1000 µg/ml imidacloprid bioassays was 1.0%, 3.9%, and 6.2% in 1995, 1996, and 1997, respectively. Geographical differences in susceptibility to imidacloprid of populations collected from cotton in 1997 were statistically significant and striking (Table 1). The most susceptible populations, such as Casa Grande and Marana exhibited 93-99% and 98-100% mean mortality in bioassays of 1.0 and 10 µg/ml imidacloprid, respectively. The least susceptible populations exhibited only 21-24% and 36-55% mean mortality in bioassays of the same concentrations (Table 1). While of obvious concern, these reductions in susceptibility have not resulted in detectable reductions in the relative efficacy of imidacloprid treatments in vegetables (Fig. 3). In summary, our results show clearly that resistance to imidacloprid is increasing, albeit slowly in Arizona.

Isolation and Characterization of Resistance to Imidacloprid

It is our objective to be proactive in managing resistance to the chloronicotinyl insecticides. This means providing growers with information regarding the buildup of resistance and how to best manage it before problems with imidacloprid become widespread in Arizona fields. One important step in this regard is to isolate resistant strains in the laboratory so that inheritance and stability of resistance and cross-resistance relationships can be elucidated.

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 select for higher levels of imidacloprid resistance in the laboratory. This has not been the case. Two years of selecting of two populations of whiteflies by rearing them on caged cotton plants treated with imidacloprid has not yielded substantial reductions in susceptibility (Fig. 4). Whereas we can not detect field populations with >25% survivorship of bioassays of 1000 µg/ml imidacloprid (Table 1), selection in the laboratory has not increased resistance beyond these levels (Fig. 4). This finding is enigmatic, since our data suggest that resistance levels are creeping upward in field populations. Once highly resistant populations are isolated, future work will focus on the stability of imidacloprid resistance, and characterization of cross-resistance relationships.

Ecosystem Study of Resistance Dynamics

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 (Dennehy and Williams 1997). This success could be short-lived, however, 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 crisis situation that brought about the emergency registrations of imidacloprid in vegetables in 1993. An important new concern is the anticipated registration of additional chloronicotinyl insecticides in Arizona. This increases the probablity of *Bemisia* resistance to the entire group of related insecticides. 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 southwestern Arizona.

The spatial dynamics and diversity of host plants of *Bemisia* are complex and highly variable within the different cotton-producing regions of Arizona. For example, cropping patterns and related insecticide use are quite different in areas of intense vegetable production of southwestern Arizona than in central Arizona, where cotton production is more abundant (Williams et al. 1997). To elucidate the impact of local cropping patterns on resistance buildup and decline we initiated a long-term study in 1995 at several sites in Arizona.

An important component of this study was relating cropping system differences between locations to imidacloprid use (selection pressure) and resistance development. Analysis of imidacloprid use data revealed that whitefly populations in southwestern Arizona were subjected to >10-times as much imidacloprid, on a regional basis, as those of central Arizona (Williams et al. 1997). Also, the imidacloprid used in southwestern Arizona comprised predominantly the systemic formulation, Admire¨, which remains active in plants for many weeks longer than the foliar formulation, Provado¨, which was of proportionally greater importance in central Arizona.

In the Dome Valley of southwestern Arizona, we detected significant reductions in whitefly susceptibility to imidacloprid through the progression of crops: from cotton in the summer of 1996 to broccoli in the fall, and then to broccoli in the spring of 1997 (Fig. 5). Susceptibility was regained in the spring melon and summer cotton populations of whiteflies. These latter crops often receive no imidacloprid treatments. Similar evaluations conducted in central Arizona revealed less dramatic changes through time (Fig. 5).

Inferring Cross-Resistance Relationships

We have shown that field populations of Arizona whiteflies varied significantly in susceptibility to imidacloprid. Knowing this, we investigated the cross-resistance relationships of the newer related compounds, acetamiprid (NI-25) and CGA-293343. Variation in susceptibility of Arizona whiteflies to acetamiprid was highly correlated (R^2=0.83) with differences in susceptibility to acetamiprid (Fig. 6). However, susceptibility to imidacloprid was a poor predictor (R^2=0.02) of susceptibility to CGA-293343 (Fig. 7). We cannot know whether these findings will reflect future cross-resistance relationships when a more powerful resistance to imidacloprid evolves in Arizona whiteflies.

Acknowledgments

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Table 1. Mortality (± std. dev.) of whitefly populations collected August-October, 1997 from Arizona cotton fields and bioassayed for susceptibility to imidacloprid (Admire®) using systemic leaf-disk bioassays. Summary statistics for 1996 and 1995 results are presented below and are from Williams et al. (1996, 1997).

	Concentration Imidacloprid (µg/ml)					
Site	**0**	**0.1**	**1**	**10**	**100**	**1000**
Buckeye	9.17 (9.8)	42.4 (19)	84.8 (8.8)	89.0 (7.5)	94.5 (6.5)	93.7 (7.2)
Casa Grande	5.18 (2.8)	54.8 (24)	99.1 (1.8)	99.6 (1.2)	99.1 (1.8)	99.5 (1.4)
Coolidge	10.1 (4.9)	38.0 (25)	87.9 (9.3)	94.3 (6.0)	98.4 (2.8)	100 (0)
Dome Valley	6.12 (8.4)	25.1 (15)	84.1 (11)	93.6 (6.9)	98.8 (2.0)	94.8 (7.4)
Gila River Basin #1	3.04 (3.3)	2.76 (4.1)	21.2 (26)	55.1 (15)	79.9 (13)	88.4 (6.0)
Gila Valley	2.49 (3.5)	8.07 (6.7)	24.1 (14)	36.4 (15)	56.1 (13)	72.6 (14)
Marana	14.2 (8.4)	66.4 (21)	93.3 (4.5)	98.0 (2.9)	98.8 (2.0)	98.8 (2.8)
Maricopa Ag. Ctr.	13.9 (9.2)	75.1 (12)	86.0 (9.7)	89.5 (5.6)	98.8 (2.7)	99.1 (1.9)
Mohave Valley	17.0 (8.2)	49.7 (9.7)	74.6 (6.2)	84.5 (9.0)	96.7 (3.8)	97.6 (3.3)
Paloma	4.71 (3.3)	7.81 (7.8)	32.7 (15)	49.0 (17)	83.1 (17)	83.3 (5.0)
Parker	6.77 (3.7)	44.2 (13)	68.4 (20)	82.5 (7.7)	96.2 (3.5)	97.4 (2.8)
Safford	10.4 (11)	59.6 (14)	74.4 (5.2)	83.1 (4.9)	92.6 (5.3)	93.8 (5.1)
Somerton	4.56 (4.2)	39.8 (12)	84.3 (6.6)	92.0 (6.7)	96.8 (4.2)	98.8 (2.7)
Yuma Valley Ag. Ctr.	6.20 (3.7)	49.1 (21)	90.6 (7.5)	96.7 (3.1)	100 (0)	99.6 (1.3)
	1997 Summary Statistics					
N	14	14	14	14	14	14
MEAN	8.1	40	72	82	92	94
MEDIAN	6.5	43	84	89	97	98
MINIMUM VALUE	2.5	2.8	21	36	56	73
STD. DEV.	4.5	22	26	20	12	7.8
	1996 Summary Statistics					
N	18	18	18	18	18	18
MEAN	9.2	50	84	92	97	97
MEDIAN	7.4	43	88	93	98	98
MINIMUM VALUE	2.5	16	62	68	87	86
STD. DEV.	5.0	24	12	7.8	3.7	3.9
	1995 Summary Statistics					
N	8	--	8	8	8	8
MEAN	7.2	--	75	93	98	99
MEDIAN	7.0	--	76	94	99	99
MINIMUM VALUE	1.2	--	40	86	93	97
STD. DEV.	4.5	--	17	5.3	2.4	1.2

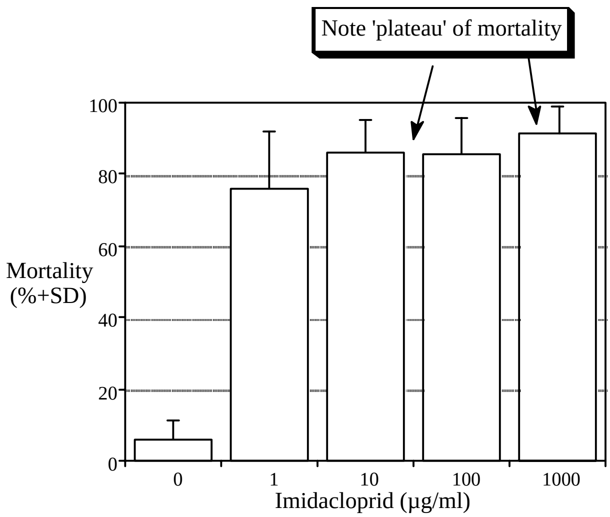

Figure 1. Baseline susceptibility to imidacloprid (Admire®) of an Arizona whitefly population collected near Yuma Arizona in 1993, prior to any exposure to this insecticide. Plateau suggests the presence of genes conferring resistance.

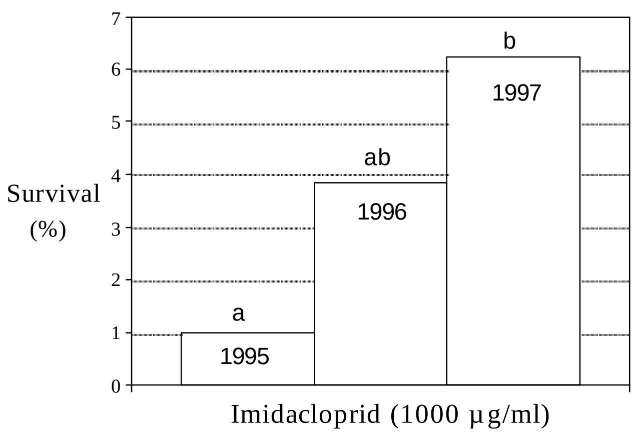

Figure 2. Reduced susceptibility of Arizona whiteflies to imidacloprid (Admire®) from 1995 to 1997. Mean survivorship across all locations has increased each of the past two years in statewide surveys.

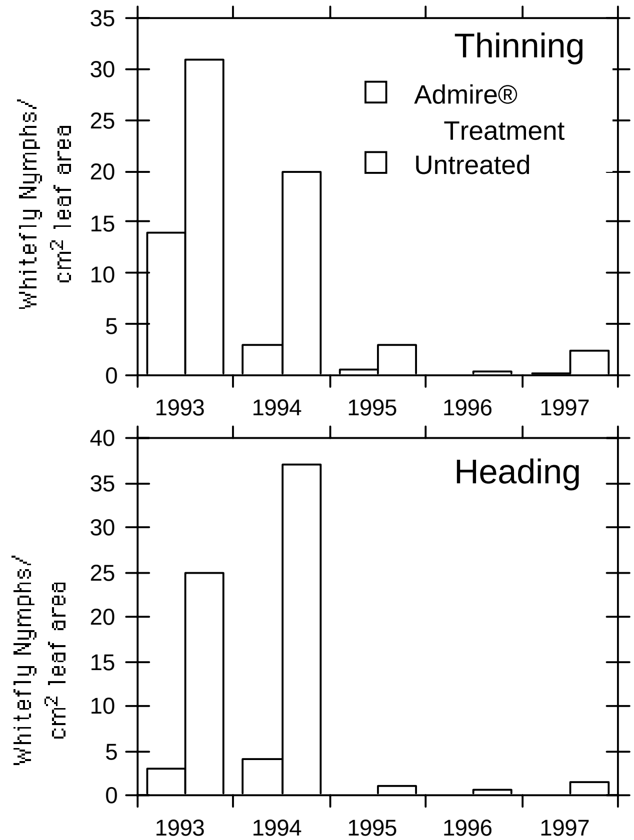

Figure 3. Relative efficacy of imidacloprid (Admire®) against whiteflies on Iceberg lettuce at Yuma, Arizona, from 1993 to 1997.

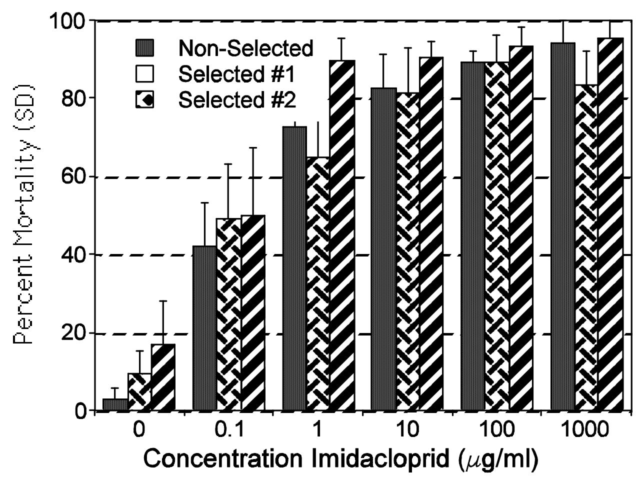

Figure 4. Whitefly susceptibility before and after approximately two years (30 generations) of selection with imidacloprid (Admire®) of two composite colonies comprised of collections from several Arizona field locations. The non-selected population was a laboratory strain maintained in the laboratory since 1993. Selection was applied by treating caged plants with Admire®.

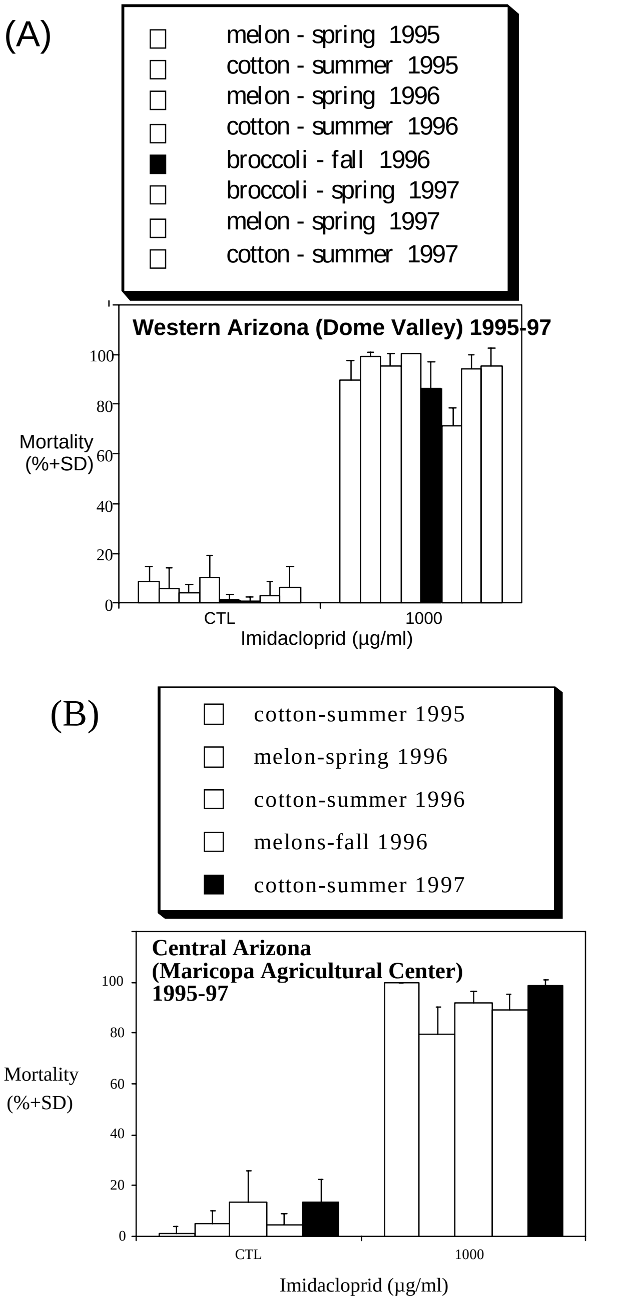

Figure 5. Contrasts of susceptibility to imidacloprid (Admire®) of whitefly populations from cropping system studies conducted at the Dome Valley (A, southwestern Arizona) and Maricopa Agricultural Center (B, central Arizona) from 1995 to 1997.

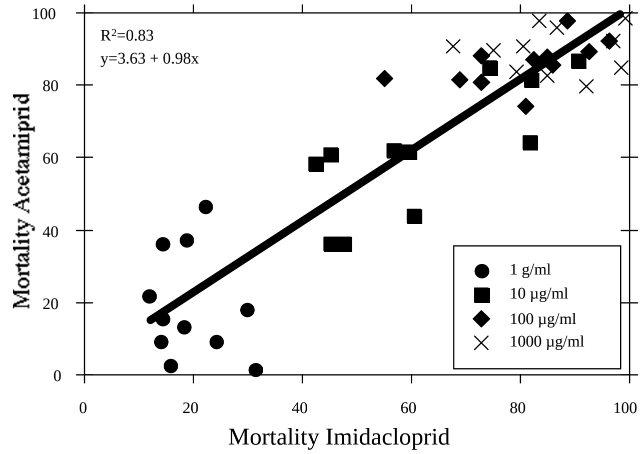

Figure 6. Relationship between susceptibility of 11 Arizona whitefly populations to imidacloprid (Admire®) versus susceptibility to the related insecticide, acetamiprid. All populations were collected from cotton fields from August-September, 1997.

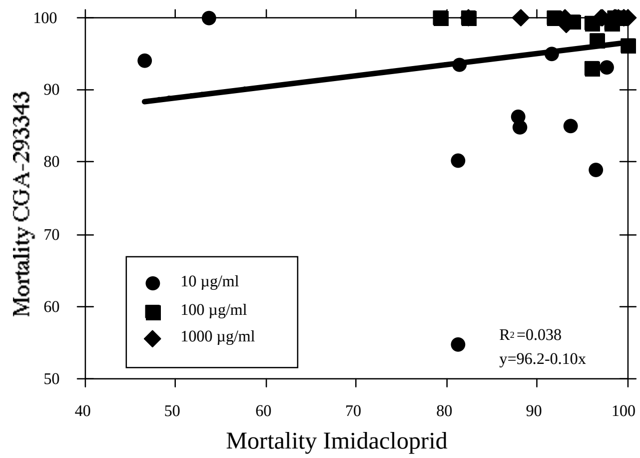

Figure 7. Relationship between susceptibility of 12 Arizona whitefly populations to imidacloprid (Admire®) versus susceptibility to the related insecticide, CGA-293343. All populations were collected from cotton fields from August-September, 1997.