SUSTAINING ARIZONA'S FRAGILE SUCCESS IN WHITEFLY RESISTANCE MANAGEMENT

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Abstract

Arizona cotton experienced a severe crisis in 1995 stemming from resistance of whiteflies to synergized pyrethroid insecticides. The insect growth regulators (IGRs), Knack® (pyriproxyfen) and Applaud® (buprofezin), served a pivotal role in resolving this problem. Similarly, Admire® (imidacloprid), the first neonicotinoid insecticide to obtain registration in Arizona, has been the foundation of whitefly control in vegetables and melons. In this paper we provide an update regarding the susceptibility to key insecticides of whiteflies from Arizona cotton, melons, and greenhouses. Overall, whitefly control in Arizona cotton remained excellent in the 2000 season and there were no reported field failures. However, there was a significant decrease in susceptibility to Applaud of whiteflies collected from cotton. One collection from Eloy, Arizona, in 2000 had susceptibility to Applaud that was reduced 129-fold relative to a reference strain. Whiteflies resistant to Knack, detected for the first time in Arizona in 1999, were again detected in 2000 but at lower frequencies than in 1999. Though whiteflies resistant to Admire/Provado® continued to be found at specific locations, overall susceptibility to Admire/Provado in 2000 remained high in whitefly collections from cotton. The new neonicotinoid insecticides, thiamethoxam and acetamiprid, were similar in toxicity to Arizona whiteflies in laboratory bioassays and we confirmed the significant but relatively low-order cross-resistance we previously reported between these neonicotinoids and Admire/Provado. Arizona whiteflies continued to be relatively susceptible to mixtures of Danitol® (fenpropathrin) and Orthene® (acephate). Factors that could undermine the current success of whitefly resistance management in Arizona are discussed. These include: 1) more severe resistance to IGRs in whiteflies from cotton, stemming from increased IGR use within and outside of cotton; 2) resistance of vegetable, melon and greenhouse whiteflies to the various formulations of imidacloprid (Admire, Provado, Merit®, Marathon®); 3) the imminent registration of new neonicotinoid active ingredients in cotton, greenhouses and other Arizona crops.

Introduction

In many areas of the desert Southwest whiteflies *Bemisia argentifolii* (a.k.a. *B. tabaci*), develop throughout the year on a succession of crop plants. In central and western Arizona they are commonly found in the fall on melons and vegetables, followed by leafy greens and Cole crops during the winter, and melons and vegetables in the spring. The numbers of whiteflies that move into cotton in the summer, and the timing of their movement, are greatly influenced by the degree to which they are controlled in these non-cotton hosts.

Arizona cotton experienced a severe crisis in 1995 stemming from resistance of whiteflies to synergized pyrethroid insecticides (Dennehy, 2000). The insect growth regulators (IGRs), Knack (pyriproxyfen) and Applaud (buprofezin), served a pivotal role in resolving this problem (Dennehy and Williams, 1997). With inputs from Israeli researchers experienced with managing whitefly resistance with IGRs (see Horowitz and Ishaaya, 1994), a three-stage chemical use strategy (Dennehy et al., 1996) was formulated for Arizona cotton. Following EPA approval for Emergency Exemptions (Section 18) for Knack and Applaud, these IGRs were recommended for use in Stage I of the new chemical use strategy, when whitefly populations first exceeded thresholds in the season. Use of each IGR was limited to once per season. Stage II comprised mixtures of non-pyrethroid conventional insecticides and Stage III comprised mixtures of pyrethroid and non-pyrethroid conventional insecticides. Since implementation in 1996, the average number of insecticide applications applied for whitefly control in Arizona cotton has declined from more than 6 in 1995 to 1 in 1999 (Dennehy, 2000, ADA 2000). Analysis of benefits of IGR adoption for whitefly control (G. Frisvold, personal communication) has shown that each application of Knack or Applaud in Arizona cotton replaced three applications of mixture of conventional insecticides. Associated with this greatly reduced use of insecticides has been regained susceptibility of whiteflies to synergized pyrethroid insecticides, endosulfan, and other insecticides (Dennehy et al., 1999).

In Arizona, long-term success of whitefly management in cotton is integrally linked to whitefly management in vegetables and melons. In this regard, Admire (imidacloprid), the first neonicotinoid insecticide to gain registration in vegetables and melons in Arizona, has been the foundation of whitefly control in these crops since 1993 (Palumbo, 1994a, b, Williams et al. 1998).

At the time of this writing there have been no confirmed field failures of Knack or Applaud in Arizona cotton, or failures of Admire in Arizona vegetables or melons. Each year we have monitored susceptibility to these insecticides on a statewide basis. From such monitoring we have documented significant declines in susceptibility of specific populations to each of these insecticides (Dennehy et al., 1999; Li et al., 2000). Moreover, research from other areas of the world has described severe resistance to these insecticides. Prabhaker et al. (1997) reported an approximately 70-fold resistance selected to Admire in the laboratory in California and control failures have been reported with this active ingredient in greenhouses in Spain (Denholm et al. 1998). Horowitz et al., (1994, 1998) have described whitefly resistance in Israel to the active ingredients of Knack and Applaud (product names are different in Israel). Horowitz and Ishaaya (1994) reported a 554-fold resistance to the active ingredient of Knack in whiteflies from a rose greenhouse in Israel and a similarly strong resistance in whiteflies from sunflower and cotton fields, despite restricted use of the chemical (Horowitz et al., 1999, Devine et al. 1999). Whiteflies resistant to the active ingredient of Applaud have been reported from greenhouses in Australia, Holland, and Israel. Up to 300-fold resistance has been attributed to repeated applications of the active ingredient of Applaud in greenhouses in Australia and Holland (De Cock et al., 1995; Cahill et al., 1996a), and control failures have been reported in Israeli greenhouses (Horowitz et al., 1998). The effectiveness of this insecticide in controlling field populations was also significantly reduced (Horowitz et al., 1998) in Israeli cotton.

Collectively, these case studies underscore the fragility of Arizona's current success in whitefly control. The re-occurrence of a major whitefly resistance event in vegetables, melons, or cotton, or as occurred in Israel, the development of resistance in greenhouses adjacent to cotton production, could disrupt whitefly control in Arizona. Clearly the recommendation of limiting use of Knack and Applaud in Arizona cotton to a maximum of one application each per season is important to sustain. However, this is not certain to prevent whitefly resistance, especially when field crops and greenhouses surrounding cotton are now using the same insecticides. Resistance to Knack's active ingredient developed in some areas of Israel even under once-per-season use (Horowitz, et al., 1998). Working collaboratively with the Arizona Cotton Growers Association, Cotton Incorporated, and the Arizona Cotton Research and Protection Council, we continue to monitor the susceptibility to key insecticides of whiteflies from Arizona cotton, melons, and greenhouses. In this paper we provide an update of our results from the 2000 season and discuss measures needed to sustain successful whitefly management in Arizona.

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Materials and Methods

Insecticides and Whitefly Populations

During 2000, we monitored susceptibility of Arizona whiteflies to Admire (imidacloprid), thiamethoxam (CGA-293343 25WG), acetamiprid (Exp-80667A), Applaud (buprofezin) and Knack (pyriproxyfen). Susceptibility to mixtures of fenpropathrin and acephate was also tested of whiteflies from cotton. Among 36 whitefly populations collected and bioassayed in 2000, fifteen were from cotton (July 10 - October 16), twelve from spring melons (April 13 - July 10), five from fall melons (August 15 - September 17), and four from Phoenix area greenhouses (August 8 - October 15). Details of collection and laboratory maintenance of whitefly populations can be found in Li et al. (2000).

IGR Bioassay Methods

Applaud® (Buprofezin). We adopted the bioassay method for buprofezin from Cahill et al. (1996b). Ten pairs of adult whiteflies were aspirated into a modified polystyrene Petri dish (OPTILUX® 100 x 15 mm) that enclosed a single cotton seedling, 14-21 days old. After the enclosed whiteflies deposited eggs for 24 h on the first true leaves of the isolated seedlings, the adults were aspirated off each seedling and the petri dish was removed from the plant, The stem of the infested leaf was then placed in a 20 ml glass scintillation vial containing tap water. The bioassays were held at 27±1˚C and a 16 h photoperiod for the duration of the assay. Eight days after the end of the oviposition period, the number of 1st instars on each leaf was recorded. Unhatched eggs were removed, and each leaf was dipped for 20 s in 50 ml of the desired concentration (0, 8, 100, or 1000 µg buprofezin/ml) of Applaud 70 WP. Mortality was determined 17 days after oviposition by counting live 3rd and 4th instars, and subtracting that number from the number of 1st instars counted on day eight on each leaf.

Knack® (Pyriproxyfen). The method for infestation of cotton leaves with whitefly eggs was the same as for the buprofezin bioassay. After the 24 hr oviposition period, adults were removed and the total number of eggs on each leaf was counted. Each infested leaf was then dipped for 20 s in 50 ml of the desired concentration (0, 0.01, 0.1, or 1 µg pyriproxyfen/ml) of Knack 0.86 EC, and allowed to dry. The stem of each infested plant was inserted individually into 20 ml glass scintillation vials containing tap water. The bioassays were held at 27±1˚C and a 16 h photoperiod for 7 days. Mortality was assessed by counting live 1st instars 7 days after dipping and subtracting this from the total number of eggs deposited on each leaf, recorded after the 24 h oviposition period.

Neonicotinoid Bioassay Methods

Admire® (imidacloprid). Since imidacloprid is used as a soil treatment for whiteflies in vegetables and melons in Arizona, we adopted a systemic-uptake bioassay, previously described by Cahill et al. (1996b). This method exposes whitefly adults to the chemical through their feeding on systemically-treated leaves (Williams et al., 1996). Cotton seedlings, G. hirsutum L. (var. DPL-50), at the second true-leaf stage of growth (18-26 days old), were cut at the mainstem soil line and their stems were placed in 200 ml of the desired concentration (0, 1, 10, 100 or 1000 µg imidacloprid/ml) of Admire 2F for 24 hrs of hydroponic uptake. Leaf disks of 2.5 cm in diameter were then excised from the true leaves and placed on a thin layer of agar gel (1.3%) in 20 ml glass scintillation vials. Ten replications (vials) were prepared for each concentration. Twenty to thirty adult whiteflies were aspirated into each vial. Vials were capped with dialysis membrane (Spectra/Por*4, Baxter Diagnostics Inc., IL), and placed in an incubator at 27±1˚C, 16L:8D light cycle for 48 h, after which the assays were read under a binocular dissecting microscope (Leica KL-750). Mortality was assessed by tapping vials on the counter and observing whitefly movement. Individuals unable to move appendages repetitively (non-reflex) were scored as dead.

Thiamethoxam and Acetamiprid. We used a leaf-dip bioassay method described by Rowland et al. (1990) for thiamethoxam and acetamiprid bioassays. Leaf disks of 2.5 cm in diameter were cut from 18-26 day old cotton plants (var. DPL-50). The leaf disks were dipped for 10 s in 50 ml of the desired concentration (0, 1, 10, 100 or 1000 µg/ml) of thiamethoxam (CGA-293343) or acetamiprid (Exp.-80667A) diluted in water. The dipped disks were allowed to dry before being placed individually on a base of agar (1.3%) in 20 ml scintillation vials. Six replications (vials) were prepared for each concentration. The remainder of the procedures, including introduction of whiteflies, assay incubation and reading, were the same as for the above-mentioned imidacloprid assays.

Synergized Pyrethroid Bioassay Method

The leaf-dip bioassay procedure used for mixtures of Danitol® (fenpropathrin) and Orthene® (acephate) was as detailed for thiamethoxam and acetamiprid. The three concentrations tested were 0, 10 and 100 µg fenpropathrin/ml, each mixed with 1000 µg acephate/ml of solution. Mortality was assessed after 48 h. Vials were tapped on the counter and individuals unable to move appendages repetitively (non-reflex) were scored as dead.

Data Analyses

Statistical differences in population responses and insecticide treatments were evaluated by ANOVA (JMP IN, SAS institute, Cary, NC). Probit analyses of the concentration-dependent mortality were undertaken using POLO-PC (LeOra Software, Berkeley, CA) to generate estimates of LC50s and slopes of the response lines, and the respective 95% fiducial limits. LC50s generated for each of the insecticides tested against each whitefly population in 2000, except imidacloprid, were subjected to a log transformation, and then used for regression analyses of cross-resistance (Neter and Wasserman, 1974). Because the LC50s for imidacloprid were below the range of concentrations tested in bioassays, regressions were conducted between the mortality observed in bioassays of 1 µg imidacloprid/ml (after transformation by arc sin square root of x) and the LC50 estimates of population susceptibility to thiamethoxam and acetamiprid. F-tests were then computed to test for significance of regressions (JMP IN).

Results and Discussions

Changes in Susceptibility of Whiteflies from Cotton to IGRs: 1996 to 2000

Applaud® (Buprofezin). We observed a decline in mean susceptibility to buprofezin of Arizona cotton whiteflies from 1996 to 1998 (Figure 1A). Susceptibility to buprofezin increased significantly in 1999, but returned to lower levels in 2000 (Figure 1A). The reasons for these changes are not understood. There was a 48% decrease in the Arizona cotton acres treated with buprofezin in 1999 compared with the previous year (ADA 2000); only 5713 cotton acres were treated with buprofezin in 2000. New registrations in melons and lettuce have recently been granted.

Mean mortality of cotton populations evaluated in buprofezin bioassays of 8 µg/ml decreased from 75.8% to 48.9% and for bioassays of 100 µg/ml, from 88.6% to 72.1%, from 1999 to 2000 (Figure 1A). More than half of the whitefly populations collected from cotton in 2000 exhibited a 10-fold reduction in susceptibility to buprofezin, based on contrasts of LC50 with that of the Somerton'93 reference strain.

Based on probit analyses, the range of LC50s of whitefly collections in 1996 was 0.26 to 0.90 µg/ml, while in 2000 LC50s ranged from 2.35 to 115.61 µg/ml. The LC50 of the Somerton'93 reference populations ranged from 0.90 to 6.15 µg/ml during this same time period. The least susceptible population collected from cotton in 2000 was from Eloy, Arizona (Figure 1B), and had a 129-fold decrease in susceptibility, based on contrasts of LC50 with the Somerton'93 strain. Whiteflies with much reduced susceptibility to buprofezin were detected in the areas of Coolidge and Maricopa Agricultural Center in both 1998 and 2000. The 2000 evaluations showed no significant differences between whiteflies collected from cotton and melons in their mean susceptibility to buprofezin.

Knack® (Pyriproxyfen). Susceptibility of whiteflies from Arizona cotton to pyriproxyfen in 2000 was not significantly different from the previous year (Figure 2), despite an increase in the mean mortality at concentrations of 0.01 and 0.1 µg/ml from 1999. During the first three years of pyriproxyfen use in Arizona, 1996-1998, no reductions in susceptibility were found in whiteflies from cotton. However, a statistically significant decrease in susceptibility was observed for the first time in the 1999 season (Figure 2). Mean mortality in bioassays of 0.01 µg/ml dropped from 84.0% in 1998 to 41.6% in 1999. Whereas it was extremely rare to have any whiteflies surviving bioassays of 0.1 µg/ml from 1996 to 1998, this changed in 1999 when the statewide grand mean was 7.69% survival of 0.1 µg/ml pyriproxyfen (Figure 2). Based on these findings we have designated 0.1 µg/ml as a diagnostic concentration for monitoring resistance to pyriproxyfen. Mean survival of statewide collections from cotton in bioassays of 0.1 µg/ml pyriproxyfen was 0.47% in 1996, 0.084% in 1997, 0.16% in 1998, 7.7% in 1999, and 0.84% in 2000 (Figure 2).

Based on probit analyses of response to pyriproxyfen, the LC50s of whitefly collections from cotton made from 1996-98 were in the range of 0.001 to 0.009 µg/ml. The LC50 of our laboratory reference population, Somerton'93, was 0.008 to 0.009 µg/ml. The whitefly population from cotton that was least susceptible to pyriproxyfen in 1999 was from the North Gila Valley, Arizona, and had an LC50 of 0.158 µg/ml (Li et al., 2000). Based on contrasts with Somerton'93, the North Gila Valley whiteflies exhibited a 19-fold resistance to pyriproxyfen. The least susceptible cotton collection in 2000 was also from North Gila Valley, Arizona, but exhibited only a 2-fold reduction in susceptibility compared to Somerton'93.

Arizona cotton acreage treated with pyriproxyfen was 143,808 in 1996, 101,842 in 1997, 115,552 in 1998, 29,676 in 1999, and 50,299 in 2000. Therefore, the first detection of resistance to pyriproxyfen, in 1999, coincided with a decline in use of pyriproxyfen. However, pyriproxyfen has been granted new registrations for use in greenhouses and field crops. No statistical differences were detected in mean susceptibilities to pyriproxyfen of whiteflies collected from cotton, melons and greenhouses in 2000. This was very different from 1999, when whiteflies from spring melons were significantly more susceptible to pyriproxyfen than were collections from cotton or fall melons (Li et al., 2000). Surprisingly, whiteflies from greenhouse ornamental plants (3 samples) in 2000 were very susceptible to pyriproxyfen.

Reductions in Use of Synergized Pyrethroids and Regained Susceptibility of Arizona Whiteflies from Cotton: 1996 to 2000

Implementation of the IGR-based resistance management strategy in 1996 coincided with dramatic decreases in the use of synergized pyrethroid insecticides (Figure 3A) and regained susceptibility of whiteflies in cotton to these insecticide mixtures (Figure 3B). Arizona's whitefly resistance management strategy recommends that pyrethroid insecticide mixtures be reserved for use against whiteflies in Stage III, late in the season. The end result of this has been that many growers have not found it necessary to use Stage III insecticides. Moreover, in the cases in which late-season whitefly control has been needed, growers have reported satisfactory efficacy of synergized pyrethroid treatments.

Unfortunately, as cotton prices have fallen, Arizona producers have been under pressure to find alternatives to the relatively expensive Knack and Applaud treatments. In some cases, this has been accomplished by returning to early-season use of synergized pyrethroids. This practice is based on a false economy. Though conventional insecticides are less expensive on a per-treatment basis, analyses of pesticide use since 1995 in Arizona cotton (G. Frisvold at the University of Arizona, personal communication) have demonstrated that each Phase I IGR treatment has replaced the equivalent of three treatments of conventional insecticides. Returning to early-season use of synergized pyrethroids in cotton, or using more than two applications of synergized pyrethroids per season, could undermine the successful management of resistance to these insecticide mixtures, and destabilize the entire whitefly resistance management success in Arizona cotton.

Regression analysis based on 2000 bioassay data revealed no cross-resistance between the synergized pyrethroids, fenpropathrin and acephate, and imidacloprid, pyriproxyfen or buprofezin used for whitefly control in Arizona. This result again underscored the value of preserving older insecticides for diversifying the insecticides used in Arizona's IGR-based whitefly resistance management program.

Changes in Susceptibility of Whiteflies to Neonicotinoid insecticides: 1995-2000

Admire®/Provado® (imidacloprid). Mean susceptibility to imidacloprid of whiteflies collected from Arizona cotton in 2000 (Figure 4A) was unchanged relative to 1999. After the striking reductions in susceptibility we reported in 1998, Arizona whiteflies have regained and sustained their susceptibility to imidacloprid in the past two years. Use of imidacloprid in cotton has been very limited, amounting to 56,663 acres treated (as Provado) in 1996, and 10,777 acres in 1997, 2063 acres in 1998, and no reported use in 1999. However, during this same period of time, imidacloprid was used intensively in Arizona vegetables, melons and greenhouses (Williams et al., 1997).

Despite the overall increase in susceptibility of whiteflies sampled from cotton, whiteflies resistant to imidacloprid continued to be found in certain locations in Arizona from 1999 to 2000. Cotton fields from Casa Blanca and Dome Valley harbored such whiteflies of low susceptibility (Figure 4B). We previously reported that whiteflies from spring melons and greenhouses had relatively lower susceptibility to imidacloprid than those from cotton and fall melons (Li et al., 2000). This was again the case in the 2000 season. The least susceptible whiteflies from spring melon fields in 2000 were, as in 1999, collected from the Dome Valley. The corrected mortality in bioassays of 1, 10, 100 and 1000 µg imidacloprid/ml was 26%, 49%, 72% and 70%, respectively, for this collection. The Dome Valley is an area of intense melon and winter vegetable production.

Thiamethoxam and Acetamiprid. Changes in cotton whitefly susceptibility to thiamethoxam and acetamiprid in Arizona followed the same general pattern as imidacloprid (Figures 4, 5A, B) from 1997 to 2000. That is, susceptibilities of whiteflies from cotton dropped to the lowest level in 1998 and then increased in the following two years. However, these changes between years were not statistically significant for thiamethoxam or acetamiprid.

In the 2000 season, no significant differences between imidacloprid, acetamiprid and thiamethoxam were found in mortality in 100 and 1000 µg/ml bioassays (Figure 6). However, imidacloprid was significantly more toxic to whiteflies than were acetamiprid or thiamethoxam at the concentrations of 1 and 10 µg/ml (Figure 6). These results confirm the 1999 observations of Li et al. (2000). Such differences in toxicity can reflect laboratory bioassay conditions and may not accurately portray difference in field performance. In particular, the systemic method used for imidacloprid may allow greater chemical uptake than the leaf-dip method used for thiamethoxam and acetamiprid. Despite differences in bioassays, it was remarkable how similar in potency all three products were against Arizona whiteflies.

Cross-Resistance Relationships of Neonicotinoid Insecticides. Regression analyses, based on the 2000 statewide survey data, revealed the existence of statistically significant cross-resistances between imidacloprid and acetamiprid ($P<0.05$) and thiamethoxam ($P<0.01$). However, the correlations were weak in both cases, with only about 16 to 26% of the variation in susceptibility to acetamiprid and thiamethoxam being explained by variation in whitefly susceptibility to imidacloprid (Figure 7A, B). A significant and stronger cross-resistance relationship was found between acetamiprid and thiamethoxam ($P < 0.001$, Figure 7C). Though similar to results we previously reported (Li et al., 2000), it should be noted that the strength of these correlations could change if new and more intense resistance mechanisms arise in Arizona whiteflies. The most important insight gained regarding cross-resistance is confirmation of earlier findings that efficacy of the new neonicotinoids is likely to be reduced to some degree in Arizona whiteflies resistant to imidacloprid and therefore, use of any of the three neonicotinoids could result in selection that increases the frequency of whiteflies resistant to all three insecticides.

Summary

Arizona cotton experienced a severe crisis in 1995 stemming from resistance of whiteflies to synergized pyrethroid insecticides. The insect growth regulators (IGRs), Knack (pyriproxyfen) and Applaud (buprofezin), served a pivotal role in resolving this problem. Similarly, Admire (imidacloprid), the first neonicotinoid insecticide to obtain registration in Arizona vegetables and melons, has been the foundation of whitefly control in these crops. Overall, whitefly control in Arizona cotton remained excellent in the 2000 season and there were no reported field failures. However, we provide clear evidence of the vulnerability of the current success to being undermined by future resistance development in cotton, melons or greenhouses.

A significant decrease in susceptibility to buprofezin was documented in whiteflies collected from cotton in 2000. One collection from Eloy, Arizona, had susceptibility to buprofezin that was reduced 129-fold, relative to a reference strain. Whiteflies resistant to pyriproxyfen, detected for the first time in Arizona in 1999, were again detected in 2000 but at lower frequencies than in 1999. Though, whiteflies resistant to Admire/Provado (imidacloprid) continued to be found at specific locations, overall susceptibility to in 2000 remained high in collections from cotton. The new neonicotinoid insecticides, thiamethoxam and acetamiprid, were similar in toxicity to Arizona whiteflies in laboratory bioassays and we confirmed the significant but relatively low-order cross-resistance between these neonicotinoids and imidacloprid. Arizona whiteflies continued to be relatively susceptible to mixtures of Danitol (fenpropathrin) and Orthene (acephate) and these mixtures continue to serve an important role in late-season whitefly control.

Factors that could threaten the future success of whitefly resistance management in Arizona include: 1) the future development of resistance to buprofezin and pyriproxyfen in whiteflies from cotton, stemming from increased use within and outside of cotton; 2) resistance of vegetable, melon and greenhouse whiteflies to the various formulations of imidacloprid (Admire, Merit, Marathon); 3) the imminent registration of new neonicotinoid active ingredients (acetamiprid and thiamethoxam) in cotton, greenhouses and other Arizona crops.

Our long-term objective is to preserve the effectiveness against whiteflies of IGRs in Arizona cotton and neonicotinoids in Arizona vegetables and melons. We have documented in Arizona the early stages of whitefly resistance to each of these insecticides. Also, we have cited specific cases in which severe whitefly resistance has impaired field performance of these insecticides outside of the US. Expanded use of IGRs and neonicotinoid insecticides against whiteflies in Arizona poses a clear threat of undermining the current success. For this reason it is extremely important that Arizona cotton growers continue to limit use of Knack and Applaud to one application each per season. Similarly, use of synergized pyrethroids should continue to be restrained. Pyrethroids, when needed, should be used only in Stage III of the resistance management strategy, late in the season.

Lastly, Arizona cotton producers may find that their best efforts in resistance management are eroded by unrestrained new uses of IGRs and neonicotinoid insecticides. For this reason it is critical to confer with melon and vegetable producers in cross-commodity coordinating committees and with State and Federal regulators, to achieve harmonization of whitefly control recommendations in Arizona's cotton, vegetable, melon agricultural ecosystem.

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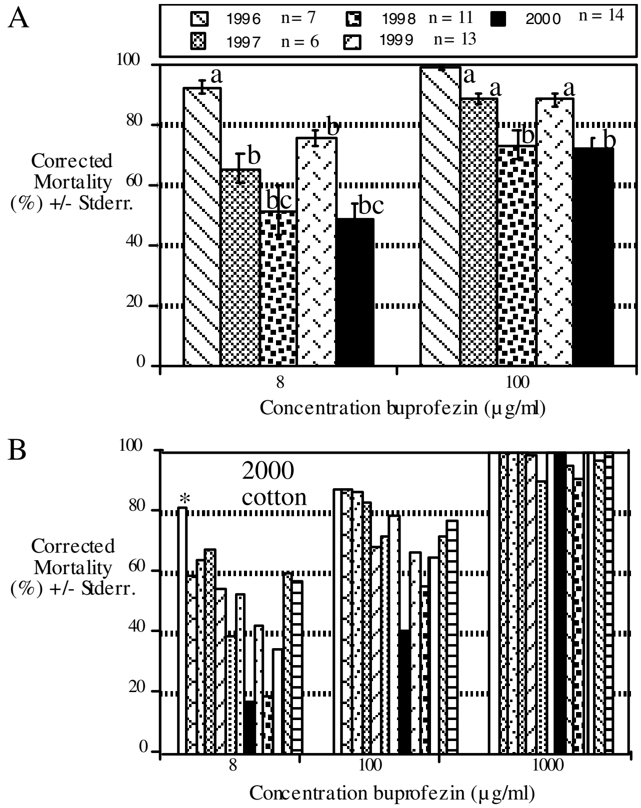

Figure 1. Susceptibility to Applaud® (buprofezin) of whiteflies collected from Arizona cotton. (A). Yearly mean (corrected) mortality observed in all cotton samples evaluated 1996-2000. Means with different letters are significantly different (ANOVA, Tukey-Kramer test, $p<0.0001$); (B). Susceptibility of specific whitefly collections evaluated in 2000. The least susceptible whiteflies were from Eloy, Arizona (black bar), which exhibited a 129-fold decrease in susceptibility to buprofezin. Somerton'93 (*) was the laboratory reference strain.

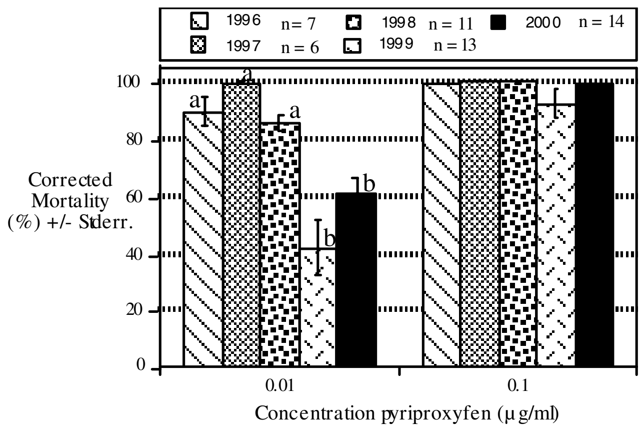

Figure 2. Susceptibility to Knack® (pyriproxyfen) of whiteflies collected from Arizona cotton: yearly mean (corrected) mortality observed in all samples evaluated 1996-2000. Survivors of diagnostic concentration bioassays of 0.1 µg/ml, first detected in 1999, were detected again in 2000 but at lower frequencies. Differences between 1999 and 2000 were not statistically significant. Means with different letters are not significantly different (ANOVA, Tukey-Kramer test, $p<0.0001$).

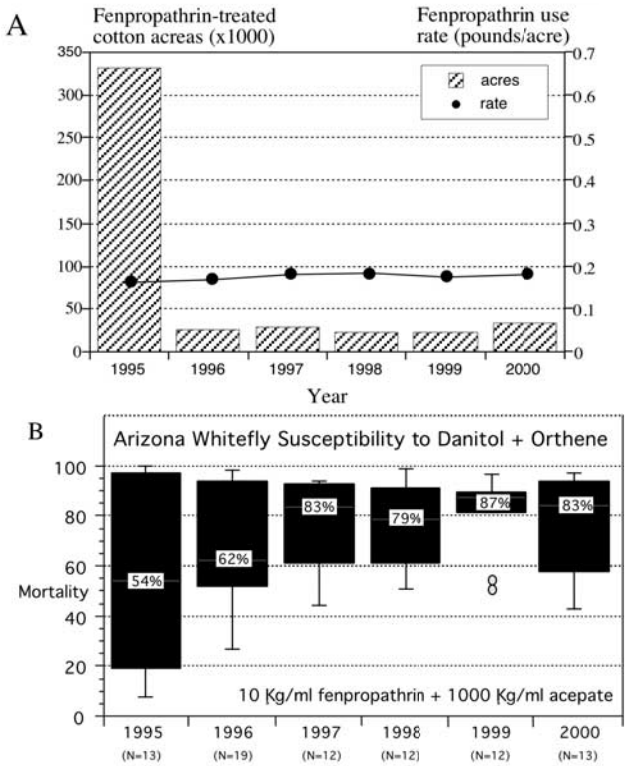

Figure 3. Reductions in the use of synergized pyrethroid insecticides, as indicated by use of mixtures of Danitol® (fenpropathrin) and Orthene® (acephate), coincided with recovery of susceptibility of Arizona whiteflies to pyrethroid mixtures. (A). The use fenpropathrin from 1995 to 2000 in Arizona cotton as determined from Form 1080 data submitted to the Arizona Department of Agriculture; (B) Changes in (corrected) mortality of whiteflies from Arizona cotton in diagnostic concentration of 10 µg/ml fenpropathrin plus 1000 µg/ml acephate. Shown are box plots of median mortality, inner quartile (boxes), and outer quartile (whiskers) values of mortality observed for all populations evaluated from 1995 to 2000.

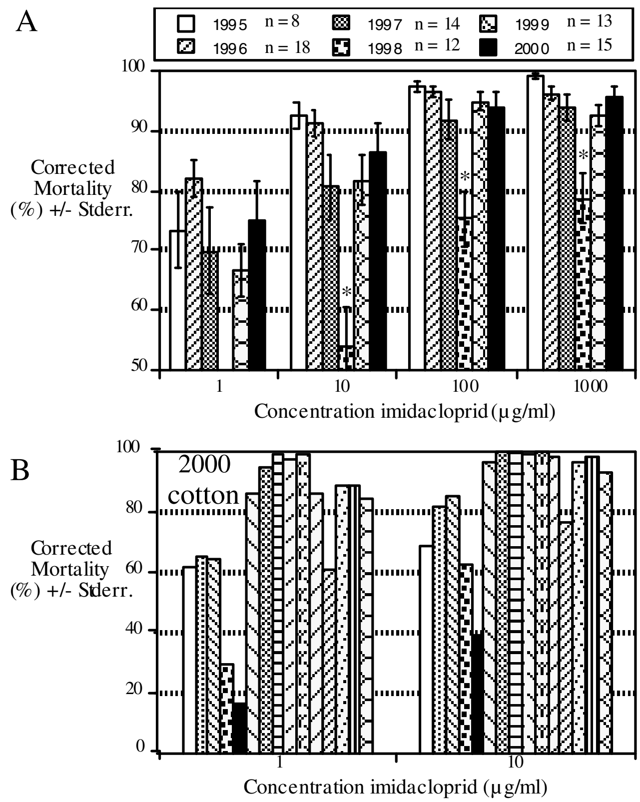

Figure 4. Susceptibility of whiteflies from Arizona cotton to imidacloprid. (A) Changes in mean (corrected) susceptibility of statewide collections from 1995 to 2000. Mean susceptibility in 1998 was significantly lower than other years at the concentrations of 10, 100 and 1000 µg/ml imidacloprid (ANOVA, Tukey-Kramer test, $p<0.001$). (B) Susceptibility of specific samples collected from Arizona cotton in 2000. The least susceptible population was from Casa Blanca (black bar).

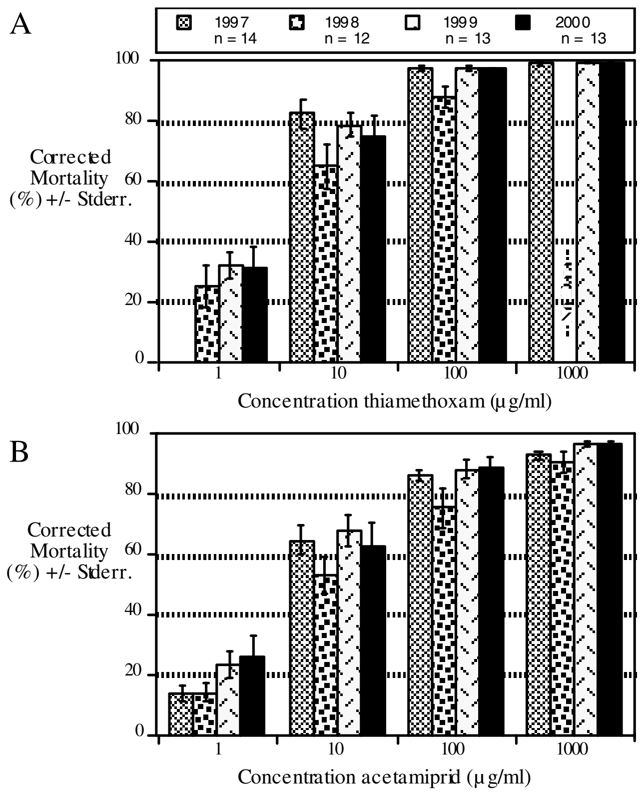

Figure 5. Susceptibility of whiteflies from Arizona cotton to two neonicotinoid insecticides soon to be registered in cotton and other crops. Shown are changes in mean (corrected) susceptibility of statewide collections from 1997 to 2000 for (A) thiamethoxam and (B) acetamiprid. No significant differences between years were found at all concentrations of either insecticide.

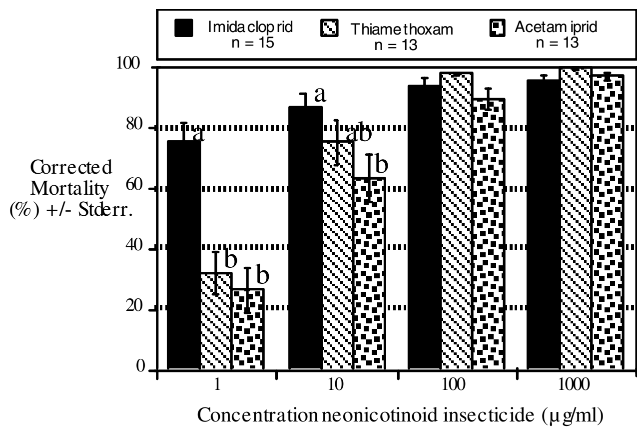

Figure 6. Relative toxicity of three neonicotinoid insecticides to whiteflies from collected from Arizona cotton in 2000 and evaluated in laboratory bioassays. Though quite similar in overall potency, the hydroponic bioassays of imidacloprid had significantly higher toxicity at lower concentrations (ANOVA, Tukey-Kramer test, p<0.0001) than did leaf-dip bioassays of thiamethoxam or acetamiprid. See Methods for details of the different bioassays.

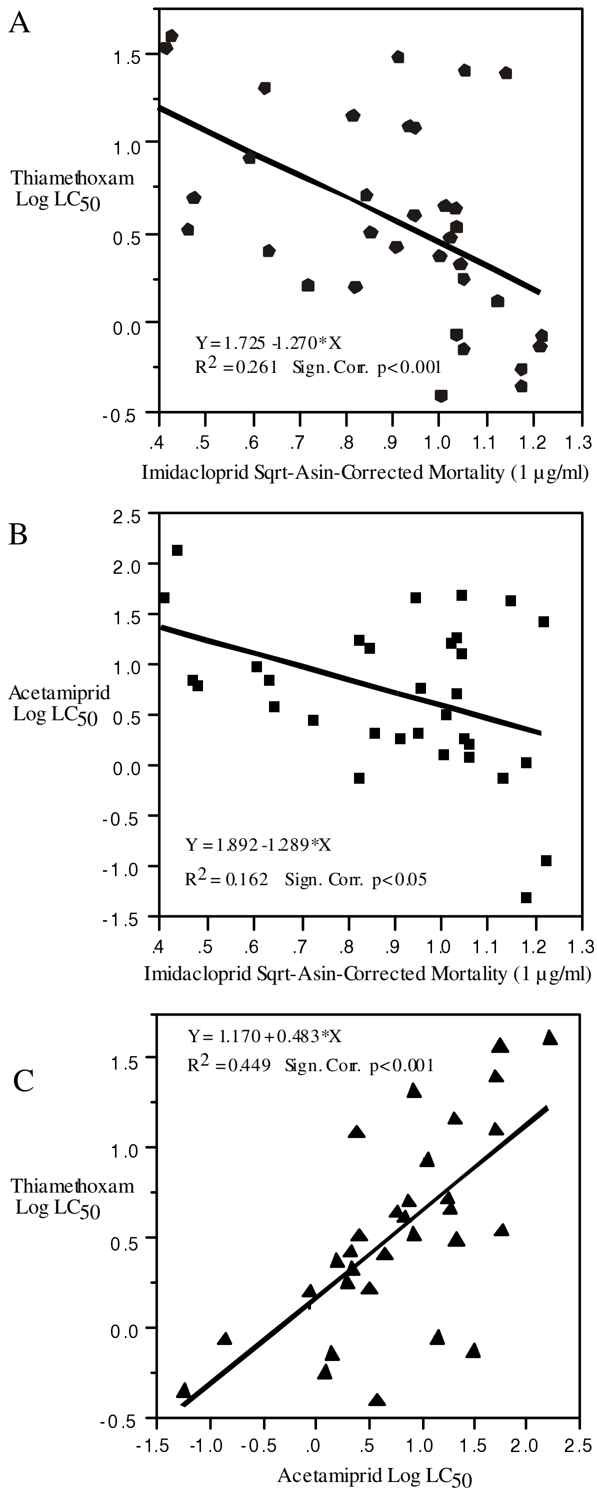

Figure 7. Bioassays of Arizona whitefly populations from cotton (N=15), melons (N=17) and greenhouses (N=4) in 2000 revealed significant but weak cross-resistance relationships between three neonicotinoid insecticides. Regression analyses were conducted on the predictor variable, mortality in bioassays of 1µg/ml imidacloprid (transformed as arcsin sqrt of X), and the response variables: (A) thiamethoxam LC50 (R2=0.261) or (B) acetamiprid LC50 (R2=0.162). (C) Significant cross-resistance was also revealed in regressions using LC50 acetamiprid as the predictor and LC50 thiamethoxam (R2=0.449) as the response variables.