

**SIX YEARS OF SUCCESSFUL MANAGEMENT OF WHITEFLY
RESISTANCE IN ARIZONA COTTON**

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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. Statewide monitoring of whitefly resistance is conducted annually in Arizona to assess the status of resistance in this important pest. In this paper we provide an update on results from whitefly collections made from 19 cotton fields located throughout Arizona. Overall, whitefly control in Arizona cotton remained excellent in the 2001 season and there were no reported field failures. However, we detected major decreases in susceptibility to Knack of whiteflies collected from cotton. Whereas it was extremely rare to have any whiteflies surviving bioassays of 0.1 µg/ml from 1996 to 1998, this changed in 1999, and by the 2001 season over 60% of Arizona sites evaluated had ≥2% pyriproxyfen-resistant whiteflies. One collection from Eloy, Arizona, in 2000 had >50% of whiteflies surviving Knack bioassays of 0.1 µg/ml. Whiteflies throughout Arizona continued to be moderately less susceptible to Applaud, relative to susceptibility levels in 1996, when the IGRs were first introduced. However, in contrast to our findings with Knack, changes in susceptibility to Applaud have been only moderate and quantitative. Arizona whiteflies continued a six year trend of reduced resistance to synergized pyrethroid insecticides, as indicated by bioassays with mixtures of Danitol and Orthene. Problematic frequencies of whiteflies resistance to synergized pyrethroids were found at only two of 19 locations sampled. Steps should be taken now to prepare for the onset of more severe resistance to IGRs in Arizona cotton. Factors that could undermine the current success of whitefly resistance management in Arizona are discussed. Education efforts should reinforce the importance of limiting IGR use in cotton to a maximum of one treatment each per season and rotating conventional insecticides as recommended in the three-stage resistance management strategy implemented in 1996. Because Knack and Applaud have received registrations for use in Arizona vegetable and melon crops grown in proximity to cotton, it is now especially critical that Extension education efforts focus on cross-commodity coordination of IGR use recommendations to preserve the activity of these important insecticides.

Introduction

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), have served pivotal roles in resolving this problem. A three-stage whitefly resistance management program, implemented in 1996, recommended that above-threshold levels of whiteflies be controlled with once-per-season use of two IGRs (Stage 1), followed by non-pyrethroid conventional insecticides (Stage 2), and, when necessary, synergized pyrethroid insecticides (Stage 3). This resistance management strategy has been shown over the past six years to be highly successful. There have been no reports of field failures of either Knack or Applaud. However, each year the UA Extension Arthropod Resistance Management Laboratory conducts extensive evaluations of whitefly resistance throughout Arizona to determine if changes in this resistance management program are needed. Herein, we provide new data for assessing the current status of, and for anticipating future challenges of, whitefly resistance to insecticides in Arizona cotton.

Materials and Methods

Collections of Whiteflies

Field populations were sampled in 2001 from 19 cotton producing areas of Arizona (Figure 1). In the field, adult whiteflies were vacuum-collected directly from cotton foliage into plastic vials using a Makita® cordless vacuum (407D). Collections were made from 50-200 plants per field, depending on whitefly density and distribution. In this manner 4,000 to 5000 adults per location were collected into each of 10-15 plastic vials. The samples were transported in ice chests directly to the Extension Arthropod Resistance Management Laboratory in Tucson, Arizona, where they were isolated in individual cages (0.5m x 0.4m x 0.6m) containing 2-4 potted cotton plants. Adult whiteflies were then aspirated from leaves of plants in these cages and placed in bioassay vials. Cages were maintained in the laboratory at 20-30°C and a 16h photophase.

Bioassay Method for Synergized Pyrethroid Insecticides

The bioassay employed was a derivative of the Rothamsted leaf disc method (Rowland et al. 1990). Leaf discs, (2.5 cm diam.) were taken from 18-26 day old cotton plants (DPL-50) and dipped for 10 s in 10 µg/ml of formulated fenpropathrin (Danitol 2.4 EC, Valent Chemical, Company, Walnut Creek, Calif.), mixed with 1000 µg/ml of formulated acephate (Orthene 75S, Valent Chemical Company) diluted with distilled water. After drying, the discs were placed individually on a base of agar within 20-ml glass scintillation vials. Within 2 h of dipping, 20-30 adult whiteflies were aspirated into each vial, vials were capped with dialysis tubing, inverted and held in an incubator at 27±0°C for 48 h, after which they were scored using a binocular dissecting microscope. Adults tested comprised approximately equal numbers of males and females. Vials were tapped on the counter 4-5 times; whiteflies not exhibiting repetitive movement of more than one appendage were scored as dead.

Levels of resistance of whiteflies to synergized pyrethroid insecticides were estimated using 10µg/ml fenpropathrin mixed with 1000 µg/ml acephate in leaf-dip bioassays. These tests, hereafter referred to as discriminating bioassays, were shown by Dennehy and Williams (1997) to provided a reliable method for detecting resistance to synergized pyrethroids in Arizona whiteflies. Field populations of whiteflies against which commercial treatments of mixtures of fenpropathrin and acephate were shown to effectively control (Simmons and Dennehy 1996) had > 90% mortality in discriminating bioassays of 10µg/ml fenpropathrin mixed with 1000 µg/ml acephate. Susceptible field strains, had >95% mortality in discriminating bioassays. Highly resistant field collections had < 10 % mortality in these bioassays (Dennehy and Williams 1997). Acephate in concentrations of as high as 1000 µg/ml did not kill highly resistant Arizona whiteflies (Dennehy et al. 1995). However, acephate strongly synergized the activity of fenpropathrin (Dennehy et al. 1995).

Applaud Bioassay Method

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) where they deposited eggs for 24 h on the first true leaves of isolated 14-21 day old cotton plants. The adults and the petri dishes were then removed, and the stem of the infested leaf was inserted 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 counted. 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/ml) of buprofezin (Applaud 70 WP, Aventis Crop Science, Research Triangle Park, NC). Survivorship was assessed 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, to determine mortality.

Knack Bioassay Method

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/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 16 h photoperiod for 7 days. Survivorship was assessed by counting live 1st instars 7 days after dipping and subtracting this from the total number of eggs deposited on each leaf, to determine mortality.

Results and Discussion

Danitol and Orthene Mixtures

Implementation of the IGR-based resistance management strategy in 1996 coincided with significant decreases in whitefly resistance to synergized pyrethroid insecticides over the following six seasons (Figure 2). Only two of 19 Arizona populations evaluated in 2001 had > 20% survivors of discriminating concentration bioassays. This contrasted sharply with 7 out of 13 collections made in 1995 that had > 20% survivors of discriminating concentration bioassays (Figure 2). Thus, it is clear that the IGR-based resistance management program has resulted in sharply reduced whitefly resistance to synergized pyrethroids. Though use of synergized pyrethroid insecticides should be reserved for late in the season, when disruption of

natural enemies is least harmful, they offer a valuable rotation product during that time of the season. Returning to early-season use of synergized pyrethroids, or using more than two applications of synergized pyrethroids per season would very likely to undermine the successful management of resistance to these insecticide mixtures, and could accelerate whitefly resistance development to both IGR and conventional insecticides in Arizona cotton.

Knack

Field reports of the performance of Knack continue to be excellent throughout Arizona cotton. However, the average susceptibility to Knack of Arizona whiteflies evaluated in statewide monitoring has declined significantly since 1999 (Figure 3a). During the first three years of pyriproxyfen use in Arizona, 1996-1998, no significant reductions in susceptibility were found in whiteflies sampled from cotton. However, a statistically significant decrease in susceptibility was observed for the first time in the 1999 season (Figure 3a, b). Whereas it was extremely rare to have any whiteflies surviving bioassays of 0.1 µg/ml from 1996 to 1998, this changed in 1999, and by the 2001 season over 60% of Arizona sites evaluated had ≥2% pyriproxyfen-resistant whiteflies (Figure 3c). The least susceptible population detected in 2001 had > 50% whiteflies surviving bioassays of 0.1 µg/ml (Figure 2c). Contrasts of five locations sampled each year from 1996-2001 revealed reductions in whitefly susceptibility at all but Buckeye, Arizona (Figure 4). Sustaining IGR effectiveness is critical to maintaining the exceptionally low insecticide use of recent years in Arizona cotton (Ellsworth and Jones 2001). Analyses by Frisvold et al. (2001) have shown that each IGR treatment replaced three treatments of conventional insecticides.

Applaud

Changes in susceptibility of Arizona whiteflies since 1996 have been only moderate and quantitative. We know of no verified failures of whitefly control with Applaud in Arizona cotton. Statistically-significant declines in mean susceptibility to Applaud have been documented in statewide monitoring from 1996 to 2001 (Figure 5a,b). The reasons for these changes are not understood. Use of Applaud has been relatively limited in Arizona cotton (Table 1). Mean mortality of statewide cotton populations evaluated in Applaud bioassays of 8 µg/ml decreased from 81.8% to 66.1% in 1996 and 2001, respectively. Mean mortality in bioassays of 100 µg/ml decreased from 98.5% to 77.3% over this same period (Figure 5a). The least susceptible of 2001 collections evaluated at this concentration were Yuma Valley Agricultural Center (56.9%), North Gila Valley (58.5%), Litchfield Park (65.5%), and Eloy (67.2%).

A Fragile Success

The six years of successful management of whitefly resistance in Arizona cotton represents a very fragile success. New registrations of IGRs in greenhouses, leafy vegetables, and melons are but one of the factors that could undermine this success. Whiteflies are highly mobile and develop year-round in Arizona on common agricultural crops and urban ornamental plants. A failure to control whiteflies or resistance problems generated in any major crop will often extend to other crops grown in the same area (Figure 6). It is for this reason that Drs. Peter Ellsworth and John Palumbo are spearheading a Cross-Commodity Coordination Committee to harmonize pest management recommendations, including whitefly control recommendations, across the major commodities in the Arizona (Palumbo et al. 1999). These efforts will be especially critical for sustaining the successful whitefly management experienced in Arizona cotton since 1996. Highest priority should be given to ensuring that Knack and Applaud are used judiciously in all crops in the system.

Conclusions

Whitefly control in Arizona cotton remained excellent in the 2001 season. There were no reported field failures of Knack (pyriproxyfen) or Applaud (buprofezin). Whitefly resistance to Danitol + Orthene has declined greatly since the implementation of the successful IGR-based resistance management program in 1996. In some areas of Arizona, whiteflies from cotton exhibited strikingly reduced susceptibility to Knack. A 2001 collection from Eloy, AZ, had over 50% resistant survivors. However, most Arizona sites tested had low frequencies of Knack-resistant whiteflies. We currently do not know the impact that this resistance has on field performance of Knack. Increased emphasis should be placed on observing field performance of Knack in the coming year. Also, contingency plans should be formulated for responding to future problems with Knack resistance. There is a critical need for harmonization of whitefly chemical control recommendations in the year-round cropping systems of the desert Southwest to avoid repeated use of IGRs in successive crops.

Acknowledgement

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Table 1. Use of IGRs in Arizona Cotton from 1996 to 2001.

Applaud[®] (buprofezin)	Treated Cotton Acres^a	Buprofezin pounds (a.i.)^a
1996	55,846	19,692
1997	67,971	23,535
1998	34,260	12,185
1999	17,921	6,367
2000	14,229	7,053
2001	29,442	14,717
Knack[®] (pyriproxyfen)	Treated Cotton Acres	Pyriproxyfen pounds (a.i.)
1996	143,808 ^a	8,499 ^a
1997	101,842 ^a	5,410 ^a
1998	115,552 ^a	6,271 ^a
1999	29,676 ^b	1,514 ^b
2000	50,299 ^b	2,673 ^b
2001	106,665 ^b	5,701 ^b

^a Section 18 Exemption required mandatory full reporting of treatments to Arizona Department of Agriculture. Data obtained from Dr. E. Minch.

^b Section 3 Registration. Mandatory reporting of treatments made by commercial applicators (est. >90% of applications) but voluntary reporting of grower-applied treatments. Data obtained from analysis of Arizona Department of Agriculture Form 1080 database.



Figure 1. Regions from which whiteflies were collected in Arizona cotton in 2001 for statewide monitoring of susceptibility to insecticides.

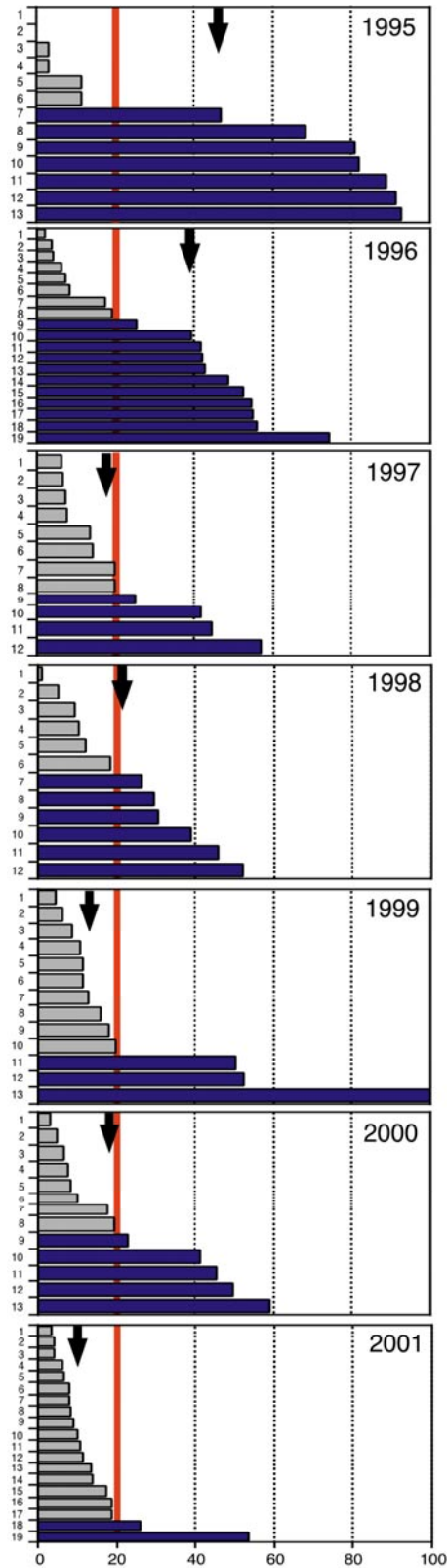


Figure 2. Reductions in resistance to synergized pyrethroid insecticides as indicated by statewide monitoring of Arizona whiteflies from 1995 to 2001. Histograms show the percent of whiteflies at each location that survived a discriminating concentration known to kill susceptible whiteflies (10 $\mu\text{g}/\text{ml}$ fenprothrin plus 1000 $\mu\text{g}/\text{ml}$ acephate). Arrows indicate median percent resistant whiteflies for each year. Dark blue bars indicate locations with $\geq 20\%$ resistant whiteflies. Synergized pyrethroids provide effective whitefly control at locations with less than this level of resistance.

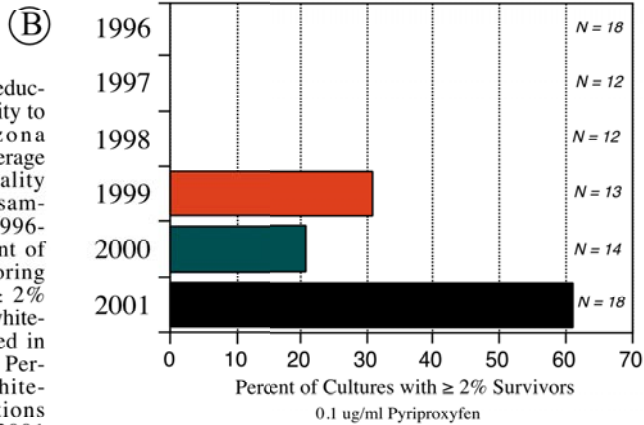
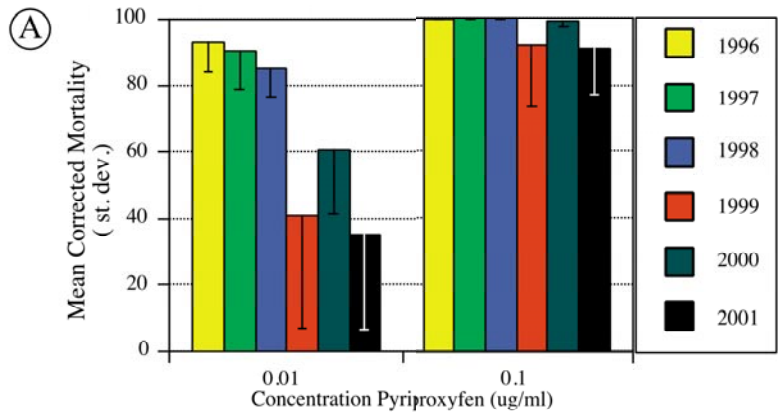


Figure 3. Slow reduction in susceptibility to Knack of Arizona whiteflies. a) Average (corrected) mortality observed in all samples evaluated 1996-2001. b) Percent of statewide monitoring sites at which $\geq 2\%$ Knack-resistant whiteflies were detected in 1996-2001. c) Percent resistant whiteflies at all locations sampled in the 2001 season.

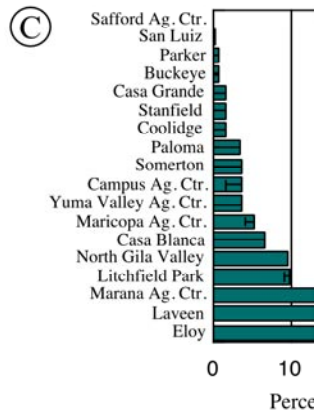


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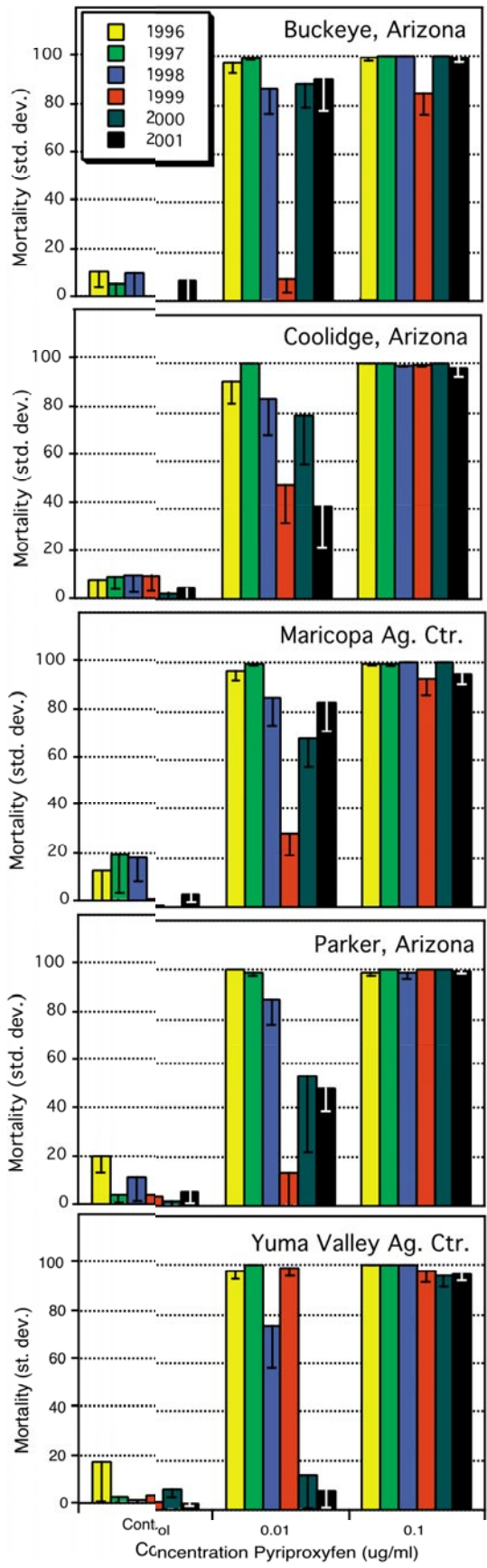


Figure 4. Changes in whitefly susceptibility to Knack at five Arizona locations monitored each year from when this IGR was first used in 1996 until 2001. All samples were collected from cotton.

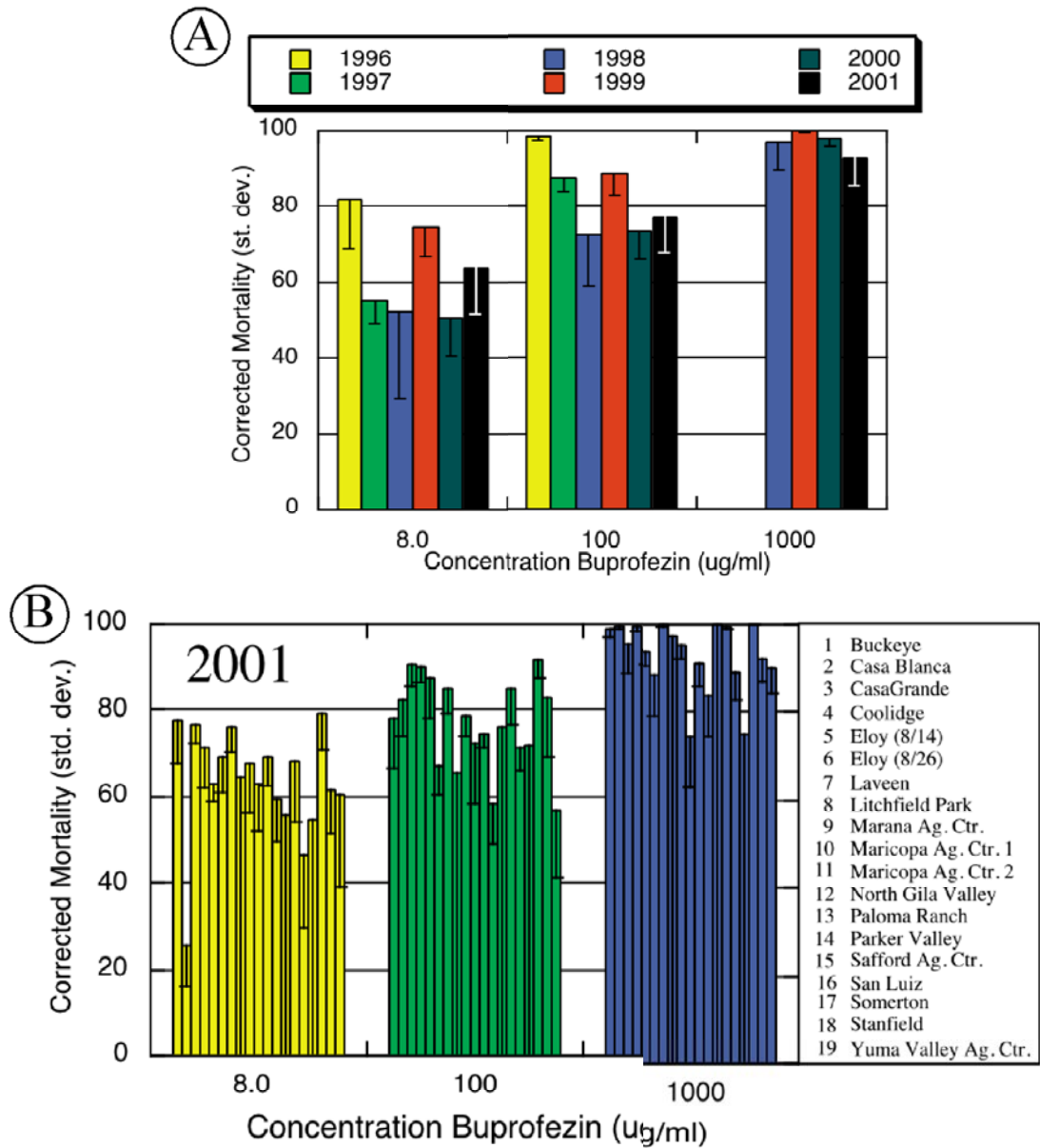


Figure 5. Whitefly susceptibility to Applaud® in Arizona cotton has declined moderately since 1996. a) Yearly mean (corrected) mortality observed in all cotton samples evaluated 1996-2001. b) Susceptibility of specific whitefly collections evaluated in 2001.

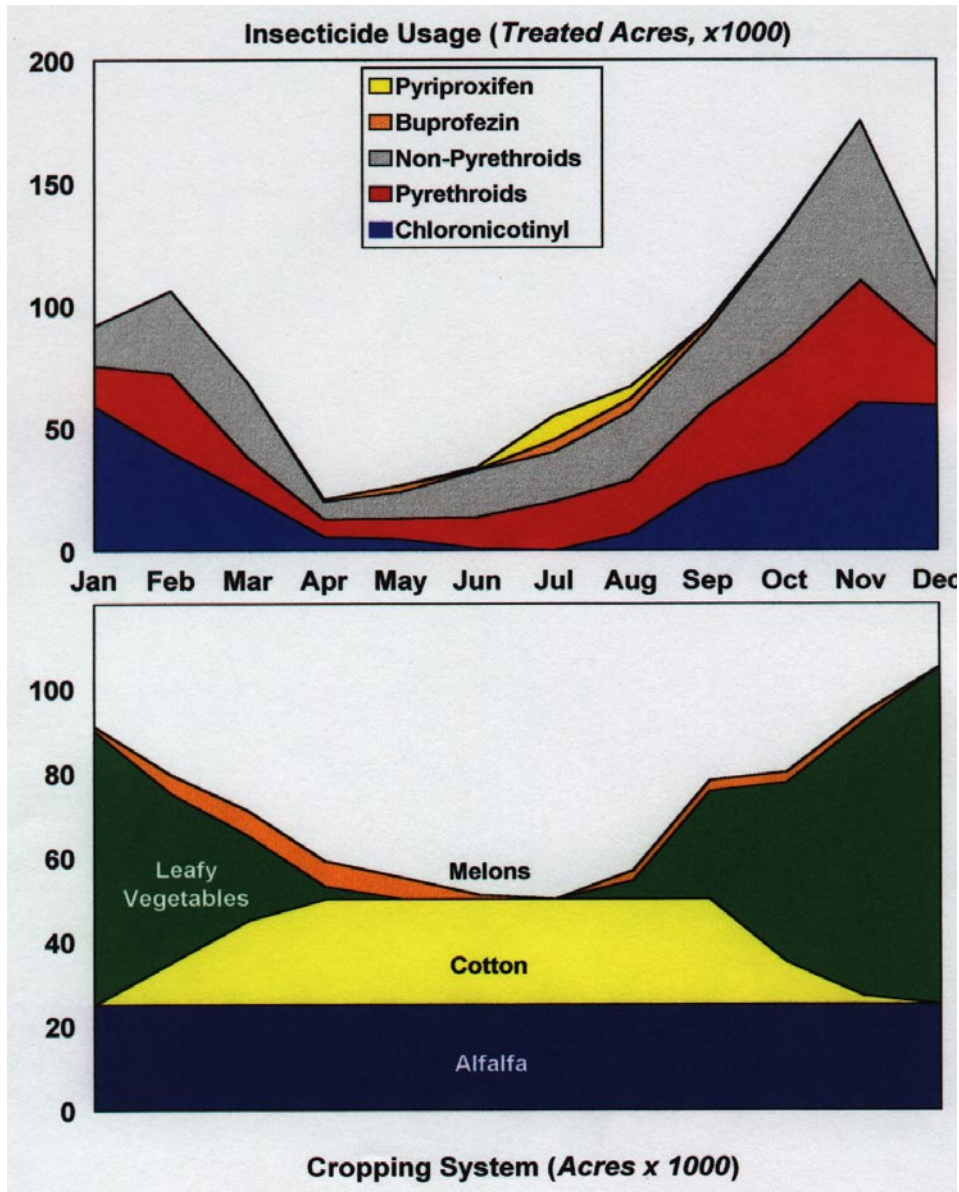


Figure 6. Estimated abundance of whitefly hosts and use of key insecticide groups used to control whiteflies in the Yuma, Arizona, region. A goal of cross-commodity coordination efforts underway in Arizona is to harmonize whitefly control recommendations within the cropping system to avoid over-reliance on specific chemical groups. *From Palumbo et al. 1999.*