INVESTIGATIONS OF TWO INSECT GROWTH REGULATORS AGAINST ARIZONA WHITEFLY POPULATIONS

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Abstract

Insect growth regulators (IGRs) served a critical role in overcoming the whitefly resistance crisis that occurred in Arizona in 1995. With the granting of a section 18 exemption in 1996, an integrated resistance management strategy was implemented to sustain the efficacy of the IGRs by using them sparingly and in the context of IPM. Bioassay methods were adapted to test susceptibility of Arizona whitefly populations to the growth regulators buprofezin (Applaud®) and pyriproxyfen (Knack®). Susceptibility of six populations was estimated early in the 1996 season prior to use of IGRs and late in the season after IGR treatments were applied to cotton. All Arizona populations were more susceptible to Applaud and Knack than were Israel whiteflies, based on published reports of Israeli findings. Significant differences were found between Arizona whitefly populations in susceptibility to Applaud and Knack during both the early and late season evaluations. Probit responses of the bulked data for all six populations early and late-season were indistinguishable for response to Applaud but significantly higher for late-season response to Knack. Field performance of both IGRs was excellent. Knack provided a minimum of 30 days whitefly suppression, while Applaud provided at least 14 days suppression. The numbers of insecticide treatments applied for whiteflies was reduced greatly in 1996, relative to 1995. Findings from the Netherlands and Israel have demonstrated the relatively rapid development of whitefly resistance to both Applaud and Knack. To sustain the impressive turn-around in whitefly control achieved with the IGRs, we recommend that Arizona cotton growers continue to limit the use to once per season, while strongly emphasizing delaying use of pyrethroids, conservation of natural enemies, and appropriate use of monitoring and thresholds.

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Introduction

In 1995, many cotton growers in Central Arizona experienced severe failure of insecticides to control whiteflies, *Bemisia argentifolii* Bellows and Perring. In problem areas, growers applied 8-12 spray applications to control whiteflies, often spending in excess of $200 per acre on insecticides. Most striking was the development of whitefly resistance to pyrethroid insecticide mixtures--pyrethroids mixed with non-pyrethroid 'synergist,' usually an organophosphate but also carbamate, cyclodiene or formamidine compounds. Prior to 1995, these so-called 'synergized pyrethroids' were the most effective insecticide treatments for controlling whiteflies in Arizona cotton. With the onset of the resistance crisis of 1995, failures of synergized pyrethroid treatments were widespread and treatments sometimes provided less than a few days of suppression of adult whiteflies.

Statewide monitoring of resistance conducted by the Extension Arthropod Resistance Management Laboratory (EARML) revealed that highly resistant whitefly populations were present at locations throughout Central Arizona but were less common in the eastern and western regions of the State. Monitoring further provided circumstantial evidence of cross-resistance between Danitol+Orthene and essentially all of the pyrethroid insecticides registered for use in Arizona cotton.

Failure of the synergized pyrethroid insecticides in Arizona cotton prompted rapid action at the end of the 1995 season on the part of the Arizona Cotton Growers Association in conjunction with University and Government pest managers and Cotton Incorporated. A new approach to whitefly control was formulated for Arizona cotton with assistance from resistance management scientists in England and Israel. The new strategy, detailed elsewhere (Dennehy et al. 1997), incorporated into the whitefly control programs three major elements: 1) registration of insect growth regulators (IGRs), Applaud® and Knack®, and limiting their use of to a maximum of once per season for each; 2) delaying use of pyrethroid insecticides for as long into the growing season as possible and thus conserving natural enemies; 3) designation of three stages of chemical use--Stage I = IGRs, Stage II = non-pyrethroids, and Stage III = pyrethroid insecticides.

The US Environmental Protection Agency granted Section 18 exemptions for Knack and Applaud in May of 1996, thereby clearing the way for implementation of the 1996 Arizona Whitefly Resistance Management Strategy. First year results of the use of these materials and implementation of the resistance management strategy were very positive. However, prior experience with these IGRs in Holland, Spain, and Israel has shown that resistance to them can be selected relatively rapidly in whiteflies. To sustain the impressive turn-around achieved with whitefly control in Arizona, cotton it is now essential that steps be taken to

combat resistance development. To that end, we describe in this paper the following fundamental building blocks of an IGR resistance management program:

1. Development of bioassay methods for Applaud and Knack.
2. Estimates of whitefly susceptibility prior to use of the IGRs in cotton.
3. Contrasting of susceptibility to IGRs before and after their use in 1996.
4. Documenting field performance of IGRs.

Materials and Methods

Chemistry - Insect Growth Regulators

Applaud 70 WP (buprofezin) is a chitin synthesis inhibitor. It is active against the nymphal stages of whiteflies. Unlike conventional chemistries, there is no acute toxicity to adults; however, treatment of adults with Applaud causes shortened adult longevity, suppression of oviposition, and egg sterility. Applaud also has a relatively high vapor pressure and has been found to volatilize and absorb to plant tissue not directly contracted at the time of spray application.

Knack 0.86 EC (pyriproxyfen) is a juvenile hormone mimic. It is very toxic to whitefly eggs due to suppression of embryogenesis. Additionally, Knack kills whiteflies in the pupal stage by inhibiting metamorphosis from the pupal to adult stages. Like Applaud, Knack has no acute activity on adults but the hatching of eggs deposited by treated adults is suppressed. Knack also exhibits translaminar movement in cotton leaves, an added benefit when managing whitefly.

Applaud Bioassay Method

The bioassay method used with Applaud was adapted from Cahill et al. (1996). 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 a cotton leaf. After the oviposition period the adults were removed and the infested leaf was placed in a 20 ml glass scintillation vial. The bioassays were held at 27°C for the duration of the assay. Eight days after the oviposition period, the number of 1st instar whiteflies on each leaf was counted and each leaf was than dipped for 20s in the desired concentration of Applaud 70 WP. Mortality of immature stages was assessed 17 days after oviposition by counting live 3rd and 4th instars and subtracting this total from the number of 1st instars present at day 8.

Knack Bioassay Method

The bioassay method for Knack was adapted from Cahill et al. (1996). Ten pairs of adult whiteflies were aspirated into a modified polystyrene Petri dish (OPTILUX® 100 x 15 mm) where they deposit eggs for 24 h on a cotton leaf. After the 24 h oviposition period, adults were removed and each infested leaf was then dipped for 20 s in the desired concentration of Knack 0.86 EC, allowed to dry and then placed in a 20 ml glass scintillation vial. The bioassays were held at 27°C for 7 days. Three days into this period, counts were made of the total number of eggs on each leaf. Mortality of eggs was assessed 7 days after dipping by counting live 1st instars and subtracting this total from the eggs counted at day 3.

Collections of Arizona Whiteflies

Early-season, prior to use of the IGRs in Arizona cotton, and late-season, following one season of use, whitefly collections were made at each of six locations in Arizona: Phoenix, Buckeye, Coolidge, Gila River Basin, Maricopa, Parker, and Yuma. Adult whiteflies were vacuum collected from field foliage using a Makita® cordless vacuum (4071D) and plastic vials with fitted screen bottoms. The samples were then transported in paper bags back to EARML, in Tucson, where they were released into cages containing young cotton plants. The whiteflies were held in culture and maintained until bioassays were conducted.

Data Analysis

Probit analyses of the concentration-dependent mortality were made using POLO-PC (Anon., 1987) to generate LC_{50} and slope values and the respective 95% fiducial limits. Additionally, this program was used to evaluate statistical significance of differences between responses of populations to Knack or Applaud.

IGR Field Trial Methodology

A trial was conducted in cotton near Gila Bend, Arizona, as part of the Gila Basin IPM Program. At this site, three insecticide treatments were evaluated--Knack, Applaud, and a conventional insecticide treatment consisting of Thiodan®+Ovasyn®. Each treatment had seven replicates arranged in a randomized complete block design. Each replicate consisted of 24 rows on a 30 inch row spacing, 1250 ft. long.

Sampling and treatment protocols followed those recommended in the 1996 Arizona Whitefly Integrated Resistance Management Strategy (Dennehy et al. 1996). Pre-treatment counts were made August 1, 1996. Treatments were applied August 2 with an AG CHEM Rogator®, and a total 10 gallon volume of water per acre. Densities of adult whiteflies were estimated from samples of 15 leaves twice per week in each replicate, using the leaf turn method described by Ellsworth et al. (1994). Densities of whitefly nymphs were also estimated each week from samples of 15 leaves per replicate (Ellsworth et al. 1996). Using a binocular microscope, counts were made of total eggs, and early- and late-instar nymphs from 1 cm diameter circles drawn near the leaf base, between the main vein and the second lateral vein of each sampled leaf.

Results and Discussion

Susceptibility to IGRs Prior to Their Use in Arizona Cotton.

All Arizona whitefly populations evaluated were highly susceptible to both IGRs (Figures 1-2), despite that they

differed widely in susceptibility to pyrethroids and other insecticides (Dennehy et al. 1997, Sivasupramaniam et al. 1997). Knack was approximately 200-500 fold more toxic than Applaud in bioassays (Table 3). At early-season, prior to the commercial use of the IGRs in Arizona cotton, LC_{50} values ranged from 0.255 to 1.91 for Applaud (Table 1) and .000280 to .00508 for Knack (Table 2). This revealed statistically significant differences in baseline responses of Arizona populations to Applaud (7-fold, $P<.001$) and Knack (20-fold, $P<.001$).

Contrasts with Susceptibility After One Season of Use

Bulking the responses of all six populations (Table 3) for early- and late-season produced virtually identical susceptibility estimates for Applaud, but revealed a statistically significant but small 2.4-fold reduction in susceptibility to Knack, based on LC_{50} values (Table 3). Despite the variability we documented in baseline responses to both IGRs and the small reduction in susceptibility to Knack from early- to late-season in 1996, all Arizona populations were substantially more susceptible to Applaud and Knack (Figures 3-4) than were susceptible whitefly populations from cotton in Israel (Ishaaya and Horowitz 1995).

Field Performance of IGRs

Intensive evaluations of the field performance of the IGRs against whiteflies in Arizona cotton were conducted in 1996 in joint USDA/University of Arizona trial (Ellsworth et al. 1997), and in various growers' fields (e.g. Lublinkhof and Comer, 1997). These data are essential for relating bioassay results such as are reported here to field performance and for documenting resistance in the future. Failure of the synergized pyrethroid combination, Danitol + Orthene, was documented in the 1995 season (Dennehy et al. 1996 and Simmons et al. 1996) and in problem locations less than 3 days of suppression of adult whiteflies was observed following treatments (Figure 5). Such results were common place, for example, in the Gila River Basin area of Central Arizona many Central Arizona growers applied 8-12 insecticide applications to control whiteflies in 1995. Introduction of the IGRs in 1996 coincided with a dramatic turn-around in whitefly control in this area. Area-wide whitefly levels were greatly reduced with the use of Applaud and Knack. Representative results of IGR applications in this area of high whitefly resistance to pyrethroids are shown in Figures 6 and 7. In all cases reported a single application provided a minimum of 14 days suppression of whiteflies with Applaud and a minimum of 30 days with Knack. Notably, the dramatic area-wide reductions achieved in 1996, coupled with some localized heavy rains that suppressed whiteflies, allowed conventional insecticides such as Thiodan + Ovasyn treatments, shown in Figures 6-7, to provide considerably better whitefly suppression than was observed in 1995. Overall, whitefly insecticide treatments were reduced to 1-4 applications in Central Arizona in 1996.

Managing Resistance to IGRs.

Arizona cotton growers have overcome the severe whitefly resistance problems they experienced in the 1995 season and the IGRs, Knack and Applaud, have been pivotal in bringing about this change. However, the newly achieved success could be short lived if resistance to the IGRs is not actively combated. Studies in Israeli cotton have shown that Applaud and Knack are of relatively high risk for resistance development in whiteflies (Horowitz & Ishaaya, 1996). Whiteflies in Israel have become highly resistant to these insecticides even when their use in cotton was limited to one application each season. For this reason, it is extremely important that Arizona cotton growers limit the use of each of these products to once per season. Doing so will not guarantee that resistance will be avoided but it should help to sustain the success of the current whitefly control strategy for 5 to 10 years. We will continue statewide monitoring of whitefly susceptibility to IGRs so that we can evaluate the merits of our resistance management program and respond most effectively to resistance once it does develop.

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Table 1. Susceptibility of Arizona whitefly to Applaud® (buprofezin) in 1996 prior to the use of IGRs (early-season) and after their use in cotton (late-season).

	Early Season		Late Season	
Location	**LC_{50}[a]**	**Slope[b]**	**LC_{50}[a]**	**Slope[b]**
Phoenix	.415	1.32	.718	1.25
upper limit	.522	(.0864)	1.24	(.109)
lower limit	.316		.324	
Buckeye	1.16	1.72	1.10	2.18
upper limit	2.07	(.191)	1.57	(.202)
lower limit	.497		.632	
Coolidge	1.91	3.74	.257	1.17
upper limit	2.68	(.348)	.521	(.158)
lower limit	1.01		.0589	
Gila Bend	1.16	1.57	.855	1.50
upper limit	2.05	(.171)	1.47	(.0760)
lower limit	.506		.417	
Maricopa	1.45	4.26	.681	1.38
upper limit	--	(.598)	.980	(.0760)
lower limit	--		.433	
Parker	.968	1.80	.621	1.13
upper limit	1.96	(.234)	.873	(.0590)
lower limit	.297		.413	
Yuma	.255	.643	.817	1.84
upper limit	.628	(.0750)	.959	(.104)
lower limit	.0482		.688	

[a] LC_{50} and 95% fiducial limits as derived from the POLO-PC program.
[b] Slope values followed in brackets by standard error

Table 2. Susceptibility of Arizona whitefly to Knack® (pyriproxyfen) in 1996 prior to the use of IGRs (early-season) and after their use in cotton (late-season).

	Early Season		Late Season	
Location	**LC_{50}[a]**	**Slope[b]**	**LC_{50}[a]**	**Slope[b]**
Phoenix	.00508	4.68	.00621	2.50
upper limit	.00566	(.229)	.00763	(.162)
lower limit	.00449		.00500	
Buckeye	.000990	2.84	.00300	1.99
upper limit	.00126	(.226)	.00592	(.0892)
lower limit	.000690		.00111	
Coolidge	.00162	2.95	.00586	6.57
upper limit	.00209	(.212)	.00667	(.740)
lower limit	.00123		.00510	
Gila Bend	.000280	1.72	.00670	2.18
upper limit	.000430	(.181)	.00860	(.160)
lower limit	.000130		.00490	
Maricopa	.00331	6.59	.00240	2.52
upper limit	.00383	(.678)	.00360	(.131)
lower limit	.00258		.00120	
Parker	.00227	2.26	.00017	1.05
upper limit	.00343	(.135)	--	(.210)
lower limit	.00109		--	
Yuma	.000700	2.34	.000300	1.11
upper limit	.000950	(.135)	--	(.220)
lower limit	.000440		--	

[a] LC_{50} and 95% fiducial limits as derived from the POLO-PC program.
[b] Slope values followed in brackets by standard error

Table 3. Bulked responses of all populations of whiteflies bioassayed against Applaud® and Knack® prior to use of the IGRs (early-season) in Arizona cotton and following one season of use (late-season).

IGR	*Collection Date*	*Slope*[a]	*LC_{50}*[a]	*95% F.L.*[a]
Applaud	**Early**	1.410	.7581	(.5679-.9713)
	Late	1.403	.7022	(.5802-.8347)
Knack	**Early**	2.236	.001380	(.0009-.0019)
	Late	2.197	.003360	(.0023-.0045)

[a] Slope, LC_{50} and 95% fiducial limits as derived from the POLO-PC program.

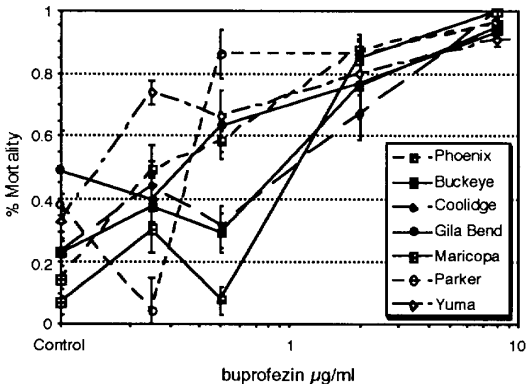

Figure 1. Susceptibility of six Arizona whitefly populations to Applaud® in 1996, prior to use of IGRs in Arizona cotton (early-season).

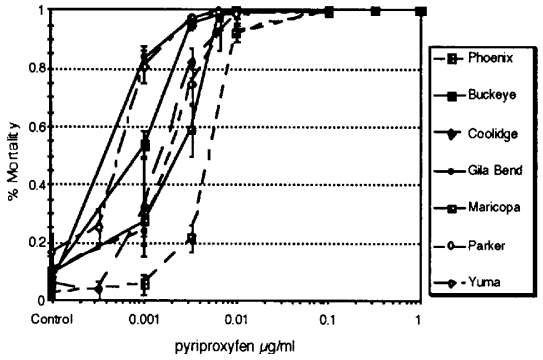

Figure 2. Susceptibility of six Arizona whitefly populations to Knack® in 1996, prior to use of IGRs in Arizona cotton (early-season).

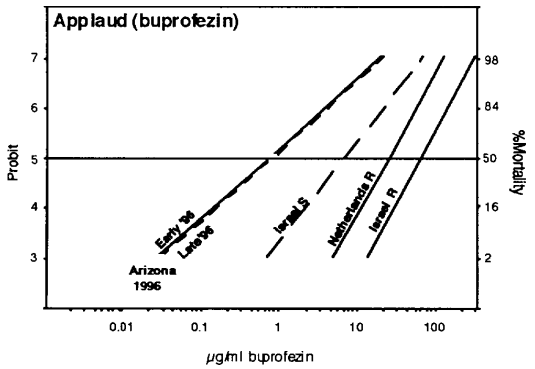

Figure 3. Responses to Applaud® of all Arizona whitefly populations evaluated in 1996 (bulked), prior to use of the IGRs in Arizona cotton (early-season) and following one season of use (late-season). Contrasts are also provided with published reports of responses to Applaud of Israel S (adapted from Ishaaya and Horowitz 1995) Netherlands R (adapted from Cahill et al. 1996), and Israel R (adapted from Horowitz 1996).

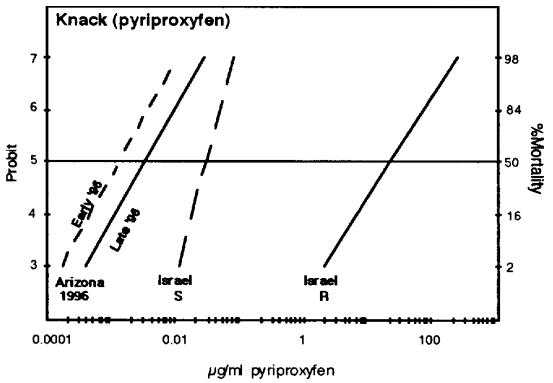

Figure 4. Responses to Knack® of all Arizona whitefly populations evaluated in 1996 (bulked), prior to use of the IGRs in Arizona cotton (early-season) and following one season of use (late-season). Contrasts are also provided with published reports of responses to Knack of Israel S and Israel R (adapted from Ishaaya and Horowitz 1995).

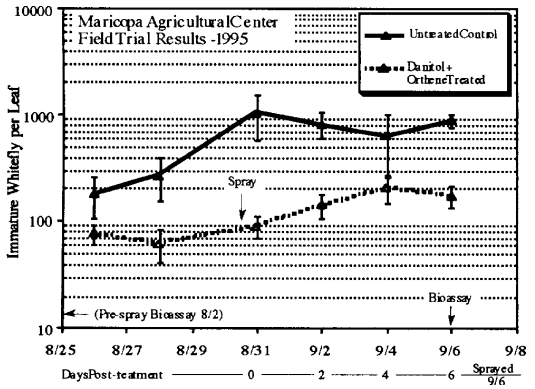

Figure 5. Severe loss of efficacy of synergized pyrethroids (Danitol® + Orthene®) against Arizona whiteflies in 1995. (From Simmons and Dennehy 1996.)

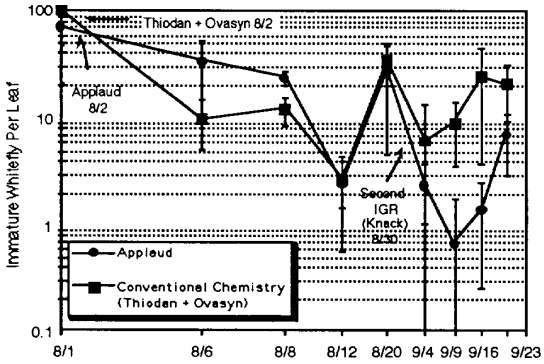

Figure 6. Efficacy of Applaud® (buprofezin) in replicated field trials conducted in 1996 by the Gila Basin IPM Program. Contrasts are shown between single applications of both Applaud® and a mixture of Thiodan® (endosulfan) + Ovasyn® (amitraz). The Applaud® plots were re-treated with a second IGR 28 days after the initial application.

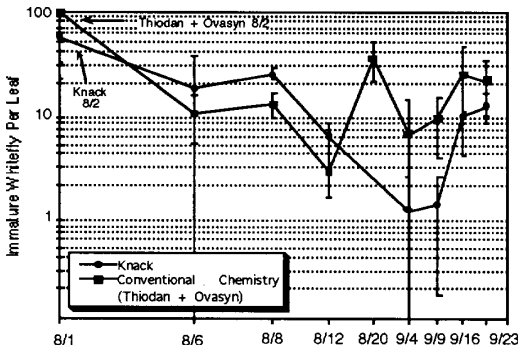

Figure 7. Efficacy of Knack® (pyriproxyfen) in replicated field trials conducted in 1996 by the Gila Basin IPM Program. Contrasts are shown between single applications of both Knack and a mixture of Thiodan® (endosulfan) + Ovasyn® (amitraz).