Pest Management Science *Pest Manag Sci* **61**:555–566 (2005)
DOI: 10.1002/ps.1030

Conservation of natural enemies in cotton: comparative selectivity of acetamiprid in the management of *Bemisia tabaci*[†‡]

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Abstract: The integrated control concept emphasizes the importance of both chemical and biological control for pest suppression in agricultural systems. A two-year field study was conducted to evaluate the selectivity of acetamiprid for the control of *Bemisia tabaci* (Gennadius) in cotton compared with a proven selective regime based on the insect growth regulators (IGRs) pyriproxyfen and buprofezin. Acetamiprid was highly effective in controlling all stages of *B tabaci* compared with an untreated control, and generally produced lower pest densities than the IGR regime. Univariate analyses indicated that nine of 17 taxa of arthropod predators were significantly depressed with the use of acetamiprid compared with an untreated control, including common species such as *Geocoris punctipes* (Say), *Orius tristicolor* (White), *Chrysoperla carnea* Stephens sensu lato, *Collops vittatus* (Say), *Hippodamia convergens* Guérin-Méneville, and *Drapetis* nr *divergens.* Compared with results from independent, concurrent studies using mixtures of broad-spectrum insecticides at the same research site, acetamiprid depressed populations of fewer predator taxa; but, for eight predator taxa significantly affected by both regimes, the average population reduction was roughly equal. In contrast, only four taxa were significantly reduced in the IGR regime compared with the untreated control and three of these were omnivores that function primarily as plant pests. Principal response curves analyses (a time-dependent, multivariate ordination method) confirmed these patterns of population change for the entire predator community. Predator:prey ratios generally increased with the use of both IGRs and acetamiprid compared with an untreated control, but ratios were consistently higher with IGRs. Parasitism by aphelinid parasitoids was unaffected or depressed slightly in all insecticide regimes compared with the control. Because of its high efficacy, acetamiprid may play an important role in later stages of *B tabaci* control where less emphasis is placed on selectivity. However, our results suggest that acetamiprid would be a poor substitute for the currently used IGRs in the initial stage of control where insecticide selectivity is crucial to a functional integrated control program for *B tabaci* in cotton.
Published in 2005 for SCI by John Wiley & Sons, Ltd.

Keywords: *Bemisia argentifolii*; arthropod predators; aphelinid parasitoids; insect growth regulators; integrated control; principal response curves

1 INTRODUCTION

Many factors may cause agricultural environments to be unsuitable for insect biological control agents, including adverse climate, scarcity of required nutrients, competition, intraguild predation, physical and chemical properties of the crop plant, lack of shelter and breeding sites, and perhaps most importantly, the indiscriminate use of insecticides.[1,2] None the less, insecticides have had and continue to play a critical role in pest control in many crops. Over forty years ago, Stern *et al*[3] formalized the integrated control concept that emphasized the importance of both chemical and biological control for pest suppression in agricultural systems. A fundamental tenet of this approach is the application of insecticides only as needed, coupled with the use of selective materials and/or selective application methods that minimize disruption of the natural enemy community.[3,4]

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) Biotype B (=*B argentifolii* Bellows & Perring) is a pest of world-wide significance causing damage to many field and horticultural crops.[5] In the southwestern USA this insect has been a been a key pest of cotton and vegetable crops since the early 1990s. Historically,

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Contract/grant sponsor: Rhone-Poulenc Ag Company, Durham, NC, USA
(*Received 19 April 2004; revised version received 5 November 2004; accepted 8 November 2004*)

B tabaci has been difficult to control with insecticides[6] and has developed resistance to many insecticide classes.[7] Much of the early effort in developing control strategies for *B tabaci* in the southern tier of the USA has focused on screening new and existing compounds for efficacy.[8,9] Subsequent research defined action thresholds for using mixtures of pyrethroids with carbamates or organophosphates,[10,11] but over-reliance on these compounds led to reduced susceptibility to pyrethroids in central Arizona by 1995.[9,12] In 1996, two insect growth regulators (IGRs), buprofezin and pyriproxyfen, were approved for use in Arizona under an emergency registration (United States Environmental Protection Agency (US-EPA), Section 18) and the use of these now fully registered compounds forms the foundation of current recommendations for whitefly and insecticide resistance management in Arizona and California.[13,14] This strategy relies on threshold-based applications of insecticides in three sequential stages beginning with selective IGRs (Stage I) followed by non-pyrethroid mixtures (Stage II) and finally pyrethroid mixtures (Stage III) near the end of the growing season. Stage II insecticides were primarily selected to enhance preservation of pyrethroid activity against *B tabaci* and less emphasis was placed on selectivity. Because of the high efficacy and selectivity of the IGRs,[15,16] this strategy ensures that the activity of natural enemies, primarily arthropod predators, is maximized during a substantial portion of the growing season. Since the introduction of this strategy in 1996, insecticide use in Arizona cotton has declined nearly 85% from 1995 to 1999.[17] The use of these IGRs along with transgenic *Bt* cotton has enabled a truly integrated control system for *B tabaci* on cotton.

None the less, even though the IGRs are generally limited to a single use each during the cotton growing season,[14] resistance to these compounds has been observed in other parts of the world[18,19] and the proactive development of alternative insecticide regimes is warranted. In the past 10–15 years several new chemistries with novel modes of action, aside from the IGRs, have been introduced for whitefly control, the most significant of these being the neonicotinoids.[9] The neonicotinoids, primarily imidacloprid, have seen widespread use in vegetables and several products are registered for use in cotton. In general, the neonicotinoids are considered to have a relatively broad spectrum of activity, although non-target exposure is thought to be minimized somewhat by their systemic and translaminar activity.[20] The neonicotinoid acetamiprid was discovered in 1989[21] and is now registered under several trade names for use on cotton in the USA.

The relatively high cost of the IGRs and other factors have led cotton growers in Arizona to consider acetamiprid as an effective alternative for the initial (Stage I) and selective control of whiteflies in place of buprofezin or pyriproxyfen (Ellsworth PC, pers comm). The effects of neonicotinoids on non-target organisms has largely focused on imidacloprid in which both foliar and systemic application method have been shown to be generally toxic[22–26] to several insect predators and parasitoids in laboratory and field residual bioassays. The few laboratory and field residual bioassay studies that have addressed the non-target effects of acetamiprid suggest that it is toxic to some natural enemies,[23,26–29] but relatively non-toxic to others.[30,31] These findings suggest that effects are species- and system-dependent and in need of further investigation. To our knowledge, no studies have examined the effects of acetamiprid on natural enemies under field conditions using threshold-based decision guidelines for insecticide application.

Controlled field studies were conducted in 1997 and 1998 to examine the comparative efficacy and selectivity of acetamiprid for control of *B tabaci* in Arizona cotton relative to a known efficacious and selective insecticide regime based on the initial use of pyriproxyfen and buprofezin. In this paper we compare the suppression of *B tabaci*, the abundance of arthropod predators, predator:ratios, and parasitism by aphelinid parasitoids between management strategies using acetamiprid and IGRs.

2 MATERIALS AND METHODS

2.1 Study site and experimental design

All studies were conducted at the University of Arizona, Maricopa Agricultural Center, Maricopa, AZ, USA. Cotton, *Gossypium hirsutum* L (cv Deltapine 5415 in 1997, Deltapine NuCOTN 33B in 1998), was planted in early to mid-April each year, and grown according to standard agronomic practices for the area.

Similar experimental designs were used in both years and consisted of a randomized complete block replicated six times in 1997 and four times in 1998. Individual plots were 12 rows (1 m centers) by 18.3 m long in 1997 and 12 rows (1 m centers) by 33.2 m long in 1998. In 1997 treatments consisted of acetamiprid 700 g kg^{-1} WP (NI-25, Rhone-Poulenc, Research Triangle Park, NC) at 56 and 84 g active ingredient (AI) ha^{-1}, a commercial standard consisting of the initial use of insect growth regulators (IGRs), and an untreated control. One additional treatment (acetamiprid at 112 g AI ha^{-1}) was added in 1998. All insecticide applications were made on the basis of weekly insect sampling (see Section 2.2). For acetamiprid applications an action threshold of five adult *B tabaci* per leaf was used.[11] For the IGR treatment, pyriproxyfen (60 g AI ha^{-1}) was initially applied at a threshold of one large nymphal whitefly (third or fourth instar) per 3.88 cm^2 leaf disk plus five adult whiteflies per leaf.[13] This was followed by the use of the IGR buprofezin (392 g AI ha^{-1}) as needed based on the same threshold, but no sooner than 3 weeks after the application of pyriproxyfen. The waiting period between IGR uses was mandated by the US-EPA Section 18 labels in force at the time. This label also permitted only a single use of each

IGR per season. If additional suppression was needed in the IGR treatment, fenpropathrin ($224\,\mathrm{g\,AI\,ha^{-1}}$) plus acephate ($561\,\mathrm{g\,AI\,ha^{-1}}$) was used based on a threshold of five adult whiteflies per leaf.[10] In 1997, applications of *Bacillus thuringiensis* var *kurstaki* ($1122\,\mathrm{g\,AI\,ha^{-1}}$) were made to all plots on 7 and 13 August to suppress an outbreak of caterpillars. In 1998, a single application of oxamyl ($843\,\mathrm{g\,AI\,ha^{-1}}$) was made in all plots on 26 June to control *Lygus hesperus* Knight. All applications were made by tractor-mounted ground sprayers (five nozzles per row) at $280\,\mathrm{liter\,ha^{-1}}$.

2.2 Arthropod sampling

Densities of *B tabaci* eggs, nymphs, and adults were estimated each week from early July through mid-September each year. Nymphal and egg densities were estimated by counting individuals (at 10× on a dissecting microscope) on a $3.88\,\mathrm{cm^2}$ disk taken from the fifth mainstem leaf below the terminal.[32] Nymphs were categorized as either small (first or second instar) or large (third or fourth instar) for the purpose of threshold implementation (see Section 2.1). Adult density was estimated by counting individuals, *in situ*, on the underside of leaves from the fifth mainstem node below the terminal.[33] Thirty sample units were randomly collected per plot for immature and adult stages on each sample date. In 1997, adult whiteflies were sampled using a binomial sampling protocol[34] in which an individual leaf was considered infested if it contained three or more adult whiteflies. Using this approach, a density of five adults per leaf was equivalent to 57% infestation. In 1998 all adults were counted on the fifth mainstem node leaf to provide better resolution of adult density, especially in the untreated control plots. Decisions to apply insecticides for *B tabaci* control were based on insect densities averaged over all six or four replicate plots in 1997 and 1998, respectively.

Arthropod predators were sampled each week with a standard 38-cm diameter sweep net from mid-June to early July through early to mid-September each year. Twenty-five sweeps were collected in each plot using a random starting point. Samples were frozen and later sorted in the laboratory with the aid of a dissecting microscope. Densities of 17 taxa of arthropod predators were estimated. Immature and adult stages of most taxa were pooled for analyses. *Lygus hesperus, Pseudatomoscelis seriatus* (Reuter) and *Spanogonicus albofasciatus* (Reuter) were included because these species may exhibit omnivorous feeding habits.[35–37] Only the predaceous larval stages of the green lacewing were counted and, following Tauber *et al*,[38] we used the designation of *Chrysoperla carnea* Stephens sensu lato for this species. Voucher specimens reside in the Department of Entomology, University of Arizona, Tucson, research collection.

Overall predator:prey ratios were calculated as the quotient of all predators combined (per 25 sweeps) to the combined number of *B tabaci* eggs, nymphs and adults per leaf. Egg and nymphal densities per leaf were estimated from regression models relating disk to whole leaf counts.[32] Densities of adults per leaf in 1997 were estimated from a regression model relating proportional infestation to density.[34]

Densities of immature aphelinid parasitoids (*Eretmocerus* spp and *Encarsia* spp) within host nymphs were estimated by taking leaf samples (30 per plot) from the seventh mainstem node below the terminal. Samples were collected weekly from early July through early to mid-September each year. In the laboratory all larval and pupal parasitoids of each genus (when possible) and all unparasitized fourth-instar whitefly nymphs on the entire leaf were counted. The presence of visible larvae or meconia within the host mummy was used to discriminate *Encarsia* spp from *Eretmocerus* spp after parasitoids reached later larval or pupal stages. Displacement of the host's mycetomes was used to determine the presence of young parasitoid larvae, but in these cases the genus of the parasitoid could not be discerned. An index of parasitism was calculated on the basis of the proportion of fourth-instar nymphs parasitized by both genera combined.

2.3 Statistical analyses

Mixed-model, repeated measures analysis of variance[39] was used to test for treatment differences over the season each year. The block variable and associated interaction terms were entered as random effects, and Satterthwaite's formula was used to estimate corrected degrees of freedom for F tests. The first-order heterogeneous autoregressive option (ARH1 in SAS Proc Mixed) was used to estimate the repeated measures covariance structure, as it consistently maximized Akaike's Information and Schwarz' Bayesian Criteria.[39] Pre-planned orthogonal contrasts were used to compare the acetamiprid treatments with the untreated control and the standard IGR treatment, to compare the IGR treatment and the control, and to contrast the two acetamiprid treatments. In 1998, this latter contrast compared the low and high rates of acetamiprid. Treatment effects on proportional parasitism were analyzed with the SAS macro, GLIMMIX,[39] which performs mixed-model ANOVA using a binomial error structure. Arthropod counts and predator:prey ratios were transformed by $(x + 0.5)^{0.5}$ or $\ln(x + 1)$ throughout as necessary to achieve normality and homoscedasticity before analyses; untransformed means are presented. Analyses were limited to sample dates following the first application of insecticides for *B tabaci*.

A meta-analysis was performed to summarize treatment effects over both years. Indices were calculated as the mean of the product $p_i s_i$ over both years, where p is the proportional reduction in density of each predator taxa, parasitism, or predator:prey ratio in a given insecticide regime relative to the untreated control in year i, and s is a dummy variable indicating the statistical significance ($s = 1$) or non-significance ($s = 0$) of the reduction based on ANOVA. In addition, mean proportional reductions (relative to the control) were calculated for all variables irrespective of statistical significance. For reference, the treatment response for an insecticide regime using a rotation of mixtures of conventional materials (pyrethroids, organophosphates, carbamates, cyclodienes) derived from a similar analysis of an independent study on the same research center in 1997 and 1998 are shown.[16]

To examine further the seasonal treatment effects on the arthropod predator community, a time-dependent, multivariate analysis called principal response curves (PRC) was conducted.[40,41] PRC is based on partial redundancy analysis, a type of principal component analysis in which information is extracted only from the variance explained by treatment effects. PRC provide a simple means of visualizing and testing the overall response of a biological community to environmental stress by determining treatment effects relative to an untreated control. The program CANOCO 4[42] was used to perform the partial redundancy analyses, construct the PRC and test for treatment differences in community composition using a distribution-free F-type test based on sample permutation. In CANOCO, the analyses can be structured to account for blocking and to allow statistical inference for individual dates or the entire season. Treatment contrasts similar to those for ANOVA above were performed. Arthropod count data were transformed by $\ln(x + 1)$ prior to analysis.

3 RESULTS

The first insecticide applications for *B tabaci* varied from late July to early August. In 1997, the following applications were made: acetamiprid (56 g AI ha^{-1}) 30 July, 29 August and 4 September; acetamiprid (84 g AI ha^{-1}) 30 July, 8 August and 4 September; and the IGR regime, pyriproxyfen 30 July, buprofezin 21 August, and fenpropathrin + acephate 4 September. In 1998 single applications of pyriproxyfen and all rates of acetamiprid were made on 6 August.

3.1 Whitefly populations

In general, populations of *B tabaci* were much higher in 1997 than in 1998 and this was reflected in the number of insecticide applications necessary to achieve control according to accepted action thresholds.[11,13] Densities of all *B tabaci* life stages were reduced by insecticide treatments compared with the untreated control in both years, with the exception that densities of eggs were not different between the IGR regime and the control in 1998 (Table 1, Fig 1). Plots treated with acetamiprid had lower densities of all stages of *B tabaci* in 1997 and lower densities of eggs and adults in 1998 compared with the IGR treatment. There were no significant differences in whitefly densities between the two rates of acetamiprid in 1997, but densities of nymphs and adults were lower in plots treated at the highest rate of acetamiprid (112 g AI ha^{-1}) compared with the lowest rate (56 g AI ha^{-1}) in 1998.

3.2 Predator populations and predator:prey ratios

Many predator taxa occurred at relatively low densities over the two years of the study, especially beetles, most spiders, and several Heteropterans. The most abundant spider was the crab spider, *Misumenops celer* (Hentz), while *Orius tristicolor* (White) and *Geocoris punctipes* (Say) were consistently the most common predaceous heteropterans. The plant pest and facultative predator *L hesperus* also consistently occurred at high densities. The empidid fly, *Drapetis* nr *divergens*, a predator of adult whiteflies, was the

Table 1. Seasonal mean densities of *Bemisia tabaci* under different control regimes, 1997–1998, Maricopa, Arizona, USA[a]

	1997			1998		
Treatment[b]	Eggs cm^{-2}	Nymphs cm^{-2}	Adults (% inf)[c]	Eggs cm^{-2}	Nymphs cm^{-2}	Adults/leaf
IGR	6.24 (±0.70)	1.18 (±0.09)	76.2 (±3.23)	4.53 (±0.88)	0.58 (±0.08)	4.01 (±0.54)
Acetamiprid (56 g ha^{-1})	5.41 (±1.05)	1.25 (±0.30)	68.7 (±2.66)	3.30 (±0.77)	0.69 (±0.08)	2.95 (±1.42)
Acetamiprid (84 g ha^{-1})	3.00 (±0.44)	0.70 (±0.11)	65.4 (±3.54)	1.91 (±0.17)	0.36 (±0.06)	0.94 (±0.30)
Acetamiprid (112 g ha^{-1})	—	—	—	2.54 (±0.64)	0.44 (±0.07)	0.8 (±0.12)
UTC	25.4 (±2.87)	8.84 (±1.20)	95.40 (±0.50)	6.29 (±0.77)	0.96 (±0.12)	6.17 (±0.35)
Orthogonal contrasts[d]						
Acetamiprid vs UTC	247**	507**	107**	36.9**	43.8**	51.9**
56 g ha^{-1} vs 84 g ha^{-1}	3.37	2.12	0.12	—	—	—
56 g ha^{-1} vs 112 g ha^{-1}	—	—	—	2.81	5.52*	8.18*
Acetamiprid vs IGR	30.5**	4.63*	9.44**	14.5**	4.40	27.9**
IGR vs UTC	69.4**	277**	35.6**	3.44	13.6**	5.21*

[a] Values are mean seasonal densities (± SEM) over seven and four post-treatment sample dates in 1997 (six replicated plots) and 1998 (four replicate plots), respectively.

[b] Insecticide rates given as grams of active ingredient per hectare. IGR regime = one application of pyriproxyfen followed by one application of buprofezin followed by one application of fenpropathrin + acephate in 1997, one application of pyriproxyfen only in 1998; UTC = untreated control.

[c] Percentage of leaves infested with ≥ three adult whiteflies.

[d] *F* values from repeated-measures ANOVA using Proc Mixed;[39] *df* estimated by Satterthwaite's correction; $P < 0.05$*; $P < 0.01$**.

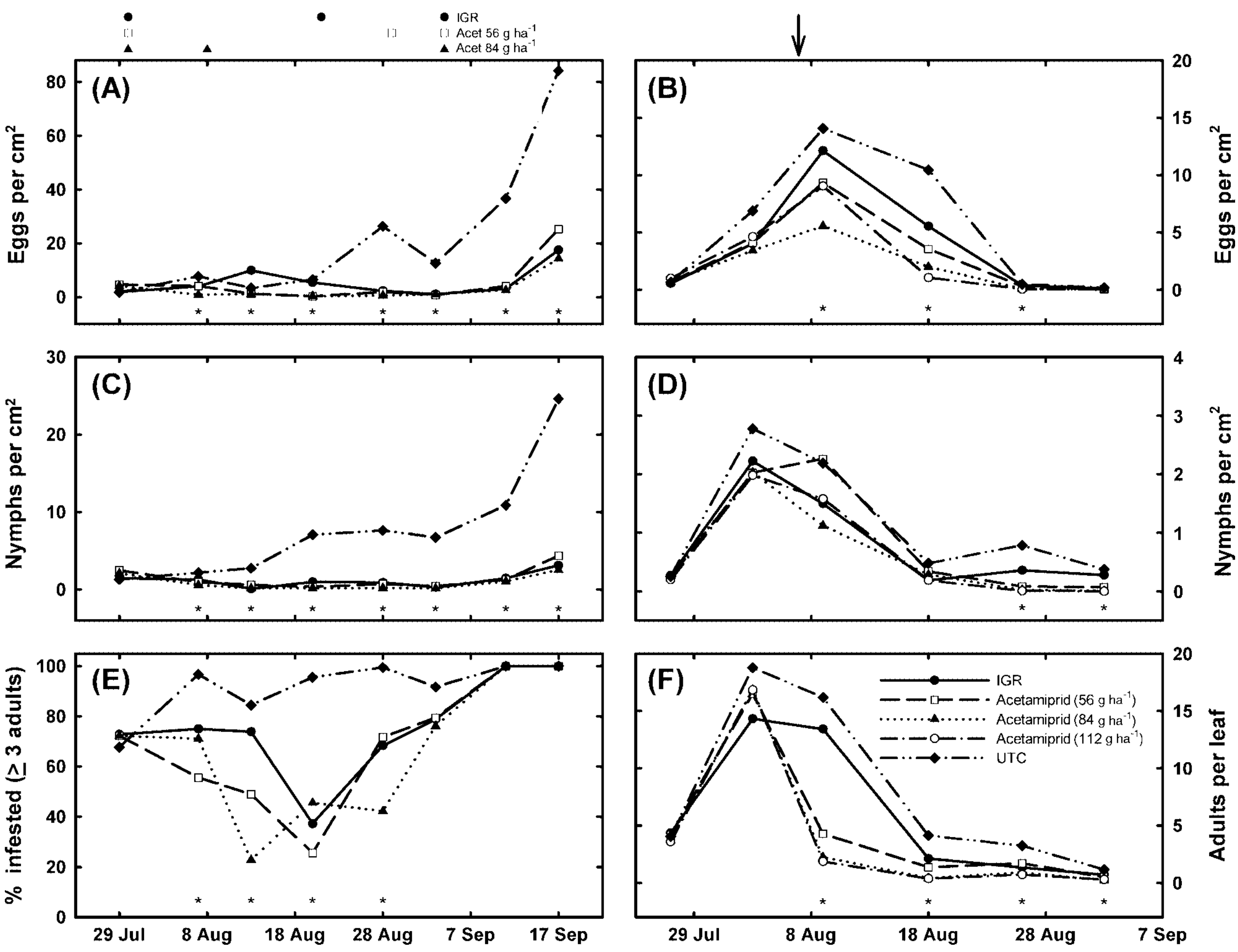

Figure 1. The effects of different control strategies on populations of *Bemisia tabaci* in (A,C,E) 1997 and (B,D,F) 1998, Maricopa, Arizona, USA. Symbols alone along the top of graph A denote the timing of insecticide applications in 1997; the arrow above graph B denotes the timing of a single application in all treatments in 1998. Asterisks along the bottom of each graph denote dates on which significant ($P < 0.05$) treatment differences were observed. Insecticide rates given as grams of active ingredient per hectare.

most abundant predator species observed over the entire study.

3.2.1 Univariate analyses

Eight predator taxa out of the 17 analyzed in 1997 were significantly reduced with the use of acetamiprid compared with the control (Table 2), including the predators *Collops vittatus* (Say), *Hippodamia convergens* Guérin-Méneville, *G punctipes, O tristicolor*, and *C carnea* sl and the omnivores *L hesperus, P seriatus* and *S albofasciatus*. Many of these taxa occurred at relatively moderate to high densities. In contrast, only *C carnea* sl, and the three omnivores above were significantly reduced in the IGR plots compared with the control. In a direct comparison of acetamiprid and the standard IGR regime, densities of *H convergens, O tristicolor* and *P seriatus* were lower in the acetamiprid plots (Table 2). The rate of acetamiprid used had no effect on densities of any predator taxa. As expected, densities of predators varied significantly ($P < 0.05$) over time in 1997 but there were only a few instances in which treatment by time interactions were observed, and in these cases interactions arose primarily from small changes in density among insecticide regimes on a few sampling dates.

Predator:prey ratios were significantly higher in the IGR plots compared with the control and acetamiprid, and higher in the acetamiprid plots compared with the control in 1997. There was no difference between rates of acetamiprid (Table 2). Predator:prey ratios varied significantly ($P < 0.05$) over time, but were generally highest in the IGR and lowest in the control plots over most sample dates (Fig 2A). The interaction between treatment and time was significant ($P < 0.05$) and this was largely a function of small changes in differences between the acetamiprid and IGR treatments over sampling dates (Fig 2A).

Overall, there were fewer treatment differences in 1998, probably due to lower whitefly pressure which limited insecticide use to only a single application in early August for all treatments. Populations of two taxa, *C vittatus* and 'other coccinellids' were too low for analysis. Three predator taxa out of the 15 analyzed were significantly reduced with the use of acetamiprid compared with the control (Table 3), including *O tristicolor, S albofasciatus* and *D* nr. *divergens*. In contrast, none of the predator taxa analyzed were

Table 2. Seasonal mean densities (per 25 sweeps) of arthropod predators, predator to prey ratios, and parasitism under different control regimes for *Bemisia tabaci*, Maricopa, Arizona, USA, 1997

	Seasonal mean (±SEM)[a,b]				Orthogonal contrasts,[c] *F* values			
Predator	IGR	Acetamiprid (56 g ha^{-1})	Acetamiprid (84 g ha^{-1})	UTC	Acetamiprid vs UTC	56 g ha^{-1} vs 84 g ha^{-1}	Acetamiprid vs IGR	IGR vs UTC
Dictyna reticulata	0.19 (±0.08)	0.11 (±0.07)	0.14 (±0.04)	0.17 (±0.06)	0.01	0.44	0.59	0.31
Misumenops celer	0.69 (±0.20)	0.89 (±0.24)	0.72 (±0.15)	0.89 (±0.23)	0.36	0.01	0.38	0.00
Jumping spiders (Salticidae)	0.11 (±0.04)	0.33 (±0.15)	0.18 (±0.05)	0.36 (±0.15)	0.31	0.85	1.29	2.16
Other spiders (Araneida)	0.21 (±0.21)	0.14 (±0.14)	0.14 (±0.14)	0.53 (±0.30)	0.93	2.78	0.34	0.11
Collops vittatus	0.14 (±0.09)	0.08 (±0.06)	0.08 (±0.04)	0.33 (±0.14)	5.16*	0.00	0.23	2.40
Hippodamia convergens	0.13 (±0.03)	0.06 (±0.02)	0.00 (±0.00)	0.17 (±0.04)	5.50*	0.50	5.73*	0.36
Other coccinellids	0.36 (±0.27)	0.25 (±0.11)	0.36 (±0.18)	0.53 (±0.40)	0.09	0.17	0.05	0.19
Geocoris punctipes	2.35 (±0.40)	2.22 (±0.48)	1.82 (±0.28)	3.75 (±0.80)	4.69*	0.15	0.15	2.37
Geocoris pallens	0.31 (±0.12)	0.39 (±0.14)	0.46 (±0.14)	0.67 (±0.29)	1.05	0.12	0.20	1.61
Orius tristicolor	5.11 (±1.17)	2.75 (±0.35)	2.18 (±0.40)	5.61 (±0.49)	14.84**	0.35	12.77**	0.06
Nabis alternatus	0.56 (±0.49)	0.11 (±0.04)	0.03 (±0.03)	0.33 (±0.07)	2.73	0.28	1.72	0.09
Zelus renardii	0.32 (±0.09)	0.44 (±0.13)	0.17 (±0.07)	0.33 (±0.16)	0.00	3.49	0.09	0.09
Lygus hesperus	6.15 (±0.66)	5.89 (±0.67)	5.24 (±0.35)	9.69 (±0.97)	30.08**	0.62	0.30	18.30**
Pseudatomoscelis seriatus	0.99 (±0.22)	0.47 (±0.17)	0.44 (±0.14)	1.97 (±0.46)	23.25**	0.01	5.36*	4.71*
Spanogonicus albofasciatus	0.11 (±0.06)	0.17 (±0.07)	0.22 (±0.07)	0.75 (±0.15)	20.92**	0.16	0.62	21.58**
Chrysoperla carnea sl	1.65 (±0.34)	1.39 (±0.27)	1.33 (±0.19)	5.00 (±0.67)	43.46**	0.06	0.25	27.81**
Drapetis nr *divergens*	25.3 (±1.42)	30.0 (±4.20)	22.3 (±1.71)	31.7 (±3.11)	2.17	4.37	0.01	1.89
Pred:prey ratio[d]	0.27 (±0.03)	0.23 (±0.03)	0.16 (±0.01)	0.08 (±0.01)	15.32**	2.04	4.81*	10.55**
Proportional parasitism[e]	0.08 (±0.02)	0.08 (±0.02)	0.06 (±0.01)	0.08 (±0.01)	1.32	1.68	0.07	1.51

[a] Seasonal means over six post-treatment sample dates in six replicate plots.

[b] Insecticide rates given as grams of active ingredient per hectare. IGR regime = one application of pyriproxyfen followed by one application of buprofezin followed by one application of fenpropathrin + acephate; UTC = untreated control.

[c] Repeated-measures ANOVA using Proc Mixed;[39] *df* estimated by Satterthwaite's correction; $P < 0.05$*; $P < 0.01$**.

[d] Quotient of all arthropod predators per 25 sweeps to *B tabaci* eggs, nymphs, and adults per leaf.

[e] Proportion of fourth-instar *B tabaci* nymphs parasitized per leaf.

affected in the IGR plots (densities of *G pallens* Stål increased relative to the control). Densities of *G pallens, O tristicolor, S albofasciatus*, and *D* nr *divergens* were lower in the acetamiprid than the IGR plots. Again, the rate of acetamiprid used had no effect on densities of any predator taxa. Densities of predators varied significantly ($P < 0.05$) over time but there were no significant ($P > 0.05$) treatment by time interactions.

Predator:prey ratios in the IGR and acetamiprid plots were similar but both were significantly ($P < 0.05$) higher than those in the control in 1998 (Table 3). Predator:prey ratios varied significantly ($P < 0.05$) over time and were highest in the IGR plots on several sampling dates (Fig 2B). There was no treatment by time interaction.

3.2.2 *Multivariate analyses*

The time-dependent effect of treatment regimes on the predator community was further examined using PRC. In 1997 the PRC based on the first axis of the redundancy analysis were highly significant ($P < 0.01$) and explained 46% of the variation due to treatment (Fig 3A). The second axis explained an additional 10% of the variance, but was not significant ($P = 0.35$). Negative canonical coefficients following insecticide applications indicate that populations of predators were generally lower in the insecticide regimes compared with the untreated control. Contrasts based on permutation tests over all sample dates combined indicated both acetamiprid and IGR treatments significantly reduced ($P = 0.014$ and 0.042, respectively) the density of the predator community compared with the untreated control. Predator densities differed significantly ($P = 0.040$) between the acetamiprid and IGR regimes, but there was no difference ($P = 0.99$) between the two rates of acetamiprid. Date by date contrasts indicated that predator densities declined significantly only on the final two sampling dates in the IGR regime following the final application of fenpropathrin plus acephate in early September. In contrast, acetamiprid caused significant reductions in predator density following the first two applications of this material in early to mid-August and also on the final two sampling dates. The species weights denote the strength of the response for each individual taxon (Fig 3A). The higher the value the more the response of a given

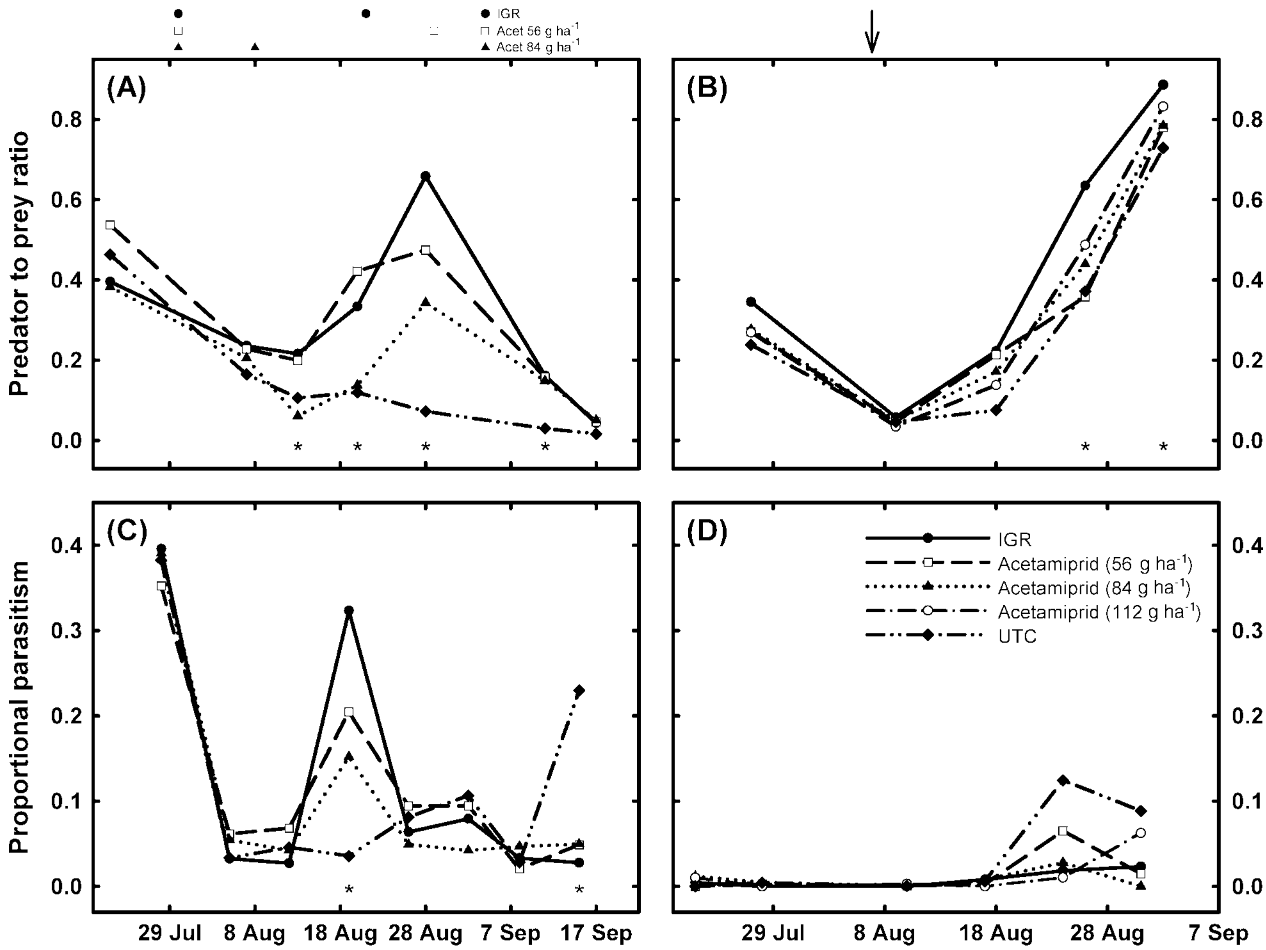

Figure 2. The effect of different strategies for control of *Bemisia tabaci* on ratios of all predators per 25 sweeps to all stages of *B tabaci* per leaf and proportional parasitism by aphelinid parasitoids, (A,C) 1997 and (B,D) 1998, Maricopa, Arizona, USA. Symbols alone along the top of graph A denote the timing of insecticide applications in 1997; the arrow above graph B denotes the timing of a single application in all treatments in 1998. Asterisks along the bottom of each graph denote dates on which significant ($P < 0.05$) treatment differences were observed. Insecticide rates given as grams of active ingredient per hectare.

taxon resembles the PRC. Negative weights indicate an opposite pattern.[41] Species weights suggest that the PRC are most representative of *C carnea* sl, *L hesperus, O tristicolor, G punctipes, D* nr *divergens, P seriatus, S albofasciatus, Nabis alternatus* Parshley and *H convergens*. In general, the product of the species weight and the canonical coefficient for a given treatment and time estimates the natural log change in density of that species relative to the control.

In 1998, PRC based on the first axis of the redundancy analysis were significant ($P = 0.008$) and explained 46% of the variation due to treatment regime (Fig 3B). The second axis explained an additional 11% of the variance, but was not significant ($P = 0.75$). Contrasts based on permutation tests over all sample dates combined indicated that acetamiprid, but not the IGR treatments, significantly reduced ($P = 0.035$ and 0.86, respectively) the density of the predator community compared with the untreated control. Predator densities were reduced in the acetamiprid compared with the IGR plots ($P = 0.023$), but again there was no difference ($P = 0.51$) between the highest and lowest rates of acetamiprid. Date by date contrasts indicated few significant differences. Predator densities declined significantly in the acetamiprid plots compared with the control on the sample date following insecticide application and again at the end of the season. Species weights suggest that the PRC are most representative of *D* nr *divergens, S albofasciatus, O tristicolor* and *G punctipes* (Fig 3B).

3.3 Parasitoid populations and parasitism

Eretmocerus spp (mainly *E eremicus* Rose and Zolnerowich) and *Encarsia* spp (mainly *E. meritoria* Gahan) were found attacking *B tabaci* at our study site. *Eretmocerus* spp were dominant, comprising 81 and 77% of all parasitoids sampled in 1997 and 1998, respectively. This generic composition was not effected by insecticide treatments but varied over time with a lower proportion (66–74%) of *Eretmocerus* earlier in the growing season and a higher proportion (>88%) later in the season. The post-treatment proportion of parasitized hosts varied widely in 1997 ranging from <0.05 on several sample dates in all treatments to >0.30 by mid-August in the IGR treatment (Fig 2C). Parasitism rates differed significantly over time but not between treatments (Table 2) and there were no interactions between treatment and sampling dates in 1997.

Table 3. Seasonal mean densities (per 25 sweeps) of arthropod predators, predator to prey ratios, and parasitism under different control regimes for *Bemisia tabaci*, Maricopa, Arizona, USA, 1998

	Seasonal mean (±SEM)[ab]					Orthogonal contrasts,[c] *F* values			
Predator	IGR[b]	Acetamiprid (56 g ha^{-1})	Acetamiprid (84 g ha^{-1})	Acetamiprid (112 g ha^{-1})	UTC	Acetamiprid vs UTC	56 g ha^{-1} vs 112 g ha^{-1}	Acetamiprid vs IGR	IGR vs UTC
Dictyna reticulata	0.13(±0.07)	0.06(±0.06)	0.13(±0.07)	0.06(±0.06)	0.13(±0.07)	0.32	0.00	1.27	1.90
Misumenops celer	6.78(±1.03)	7.06(±0.77)	6.44(±1.03)	6.31(±1.13)	7.56(±0.72)	0.52	0.45	0.20	0.05
Jumping spiders (Salticidae)	0.38(±0.16)	0.44(±0.19)	0.44(±0.06)	0.63(±0.24)	0.63(±0.16)	0.68	0.53	0.32	1.29
Other spiders (Araneida)	0.19(±0.06)	0.19(±0.12)	0.44(±0.19)	0.13(±0.13)	0.38(±0.13)	1.20	0.70	0.51	0.10
Hippodamia convergens	0.25(±0.18)	0.13(±0.13)	0.06(±0.06)	0.06(±0.06)	0.31(±0.19)	1.46	0.06	0.81	0.06
Geocoris punctipes	1.16(±0.22)	0.81(±0.28)	0.63(±0.26)	0.81(±0.12)	1.75(±0.76)	3.71	0.07	0.74	0.76
Geocoris pallens	0.44(±0.11)	0.13(±0.07)	0.06(±0.06)	0.13(±0.13)	0.25(±0.09)	0.74	0.00	5.65*	3.11
Orius tristicolor	6.75(±0.84)	5.13(±1.50)	5.75(±1.40)	5.44(±1.29)	9.00(±1.35)	13.74**	0.77	5.12*	1.94
Nabis alternatus	0.09(±0.09)	0.06(±0.06)	0.00(±0.00)	0.00(±0.00)	0.06(±0.06)	0.41	0.61	1.39	0.20
Zelus renardii	0.69(±0.21)	0.38(±0.16)	0.44(±0.21)	0.31(±0.16)	0.56(±0.16)	0.04	0.94	1.22	1.15
Lygus hesperus	23.1(±3.65)	19.9(±3.94)	19.1(±4.00)	17.6(±3.87)	20.2(±4.65)	0.27	0.79	2.72	0.85
Pseudatomoscelis seriatus	0.38(±0.16)	0.88(±0.22)	0.63(±0.24)	0.50(±0.35)	0.69(±0.24)	0.03	0.88	0.44	0.47
Spanogonicus albofasciatus	0.88(±0.26)	0.38(±0.14)	0.38(±0.20)	0.31(±0.12)	1.13(±0.30)	8.42*	0.01	5.28*	0.79
Chrysoperla carnea sl	1.09(±0.26)	0.81(±0.12)	1.19(±0.28)	0.50(±0.23)	1.13(±0.46)	3.22	0.33	0.15	3.17
Drapetis nr *divergens*	3.34(±0.43)	2.63(±0.74)	1.75(±0.27)	1.56(±0.34)	4.38(±1.15)	12.99**	1.15	5.70*	0.99
Pred:prey ratio[d]	0.41(±0.02)	0.37(±0.04)	0.35(±0.03)	0.34(±0.05)	0.30(±0.06)	6.15*	0.52	2.95	6.12*
Proportional parasitism[e]	0.01(±0.01)	0.02(±0.01)	0.01(±0.01)	0.02(±0.02)	0.05(±0.02)	9.12**	0.15	0.00	6.85*

[a] Seasonal means over four post-treatment sample dates in four replicate plots.

[b] Insecticide rates given as grams of active ingredient per hectare. IGR regime = one application of pyriproxyfen; UTC = untreated control.

[c] Repeated-measures ANOVA using Proc Mixed;[39] *df* estimated by Satterthwaite's correction; $P < 0.05$*; $P < 0.01$**.

[d] Quotient of all arthropod predators per 25 sweeps to *B tabaci* eggs, nymphs and adults per leaf.

[e] Proportion of fourth-instar *B tabaci* nymphs parasitized per leaf.

Overall, rates of parasitism were lower in 1998, barely exceeding 0.1 in late August in the untreated control plots (Fig 2D). In 1998, parasitism rates differed significantly over time and were significantly lower in plots treated with acetamiprid and the IGR pyriproxyfen compared with the control (Table 3). There were no differences among the insecticides or between the high and low rates of acetamiprid, and there was no interaction between treatment and time.

3.4 Overall impact of insecticides

Indices were calculated on the basis of statistically significant changes in seasonal densities of each predator taxon, rates of parasitism and predator:prey ratios relative to the untreated control to summarize results from both years (Table 4). Nine taxa of predators were significantly reduced by the use of acetamiprid relative to the control in at least one year. By comparison, most of these same taxa plus four additional ones were significantly reduced by a conventional insecticide regime in an independent, concurrent study at the same research site.[16] Acetamiprid depressed populations of fewer predator taxa compared with the conventional regime, but for eight predator taxa significantly affected by both regimes, the average population reduction was roughly equal. In contrast, populations of only four taxa significantly declined in the IGR regime and in all cases the reductions were smaller than with acetamiprid (Table 4). Mean predator population densities in insecticide regimes viewed as a proportion of the untreated control (Table 4, values in parentheses) further emphasize the non-selective nature of acetamiprid compared with the IGRs. Predator:prey ratios increased with the use of both IGRs and acetamiprid relative to the control although the increases were consistently higher for the IGR plots. Rates of parasitism were low overall, but declined to about the same extent with use of acetamiprid and IGRs.

4 DISCUSSION

Management strategies for *B tabaci* in the western USA have continually adapted as new sampling methods, thresholds, insecticides and resistance management

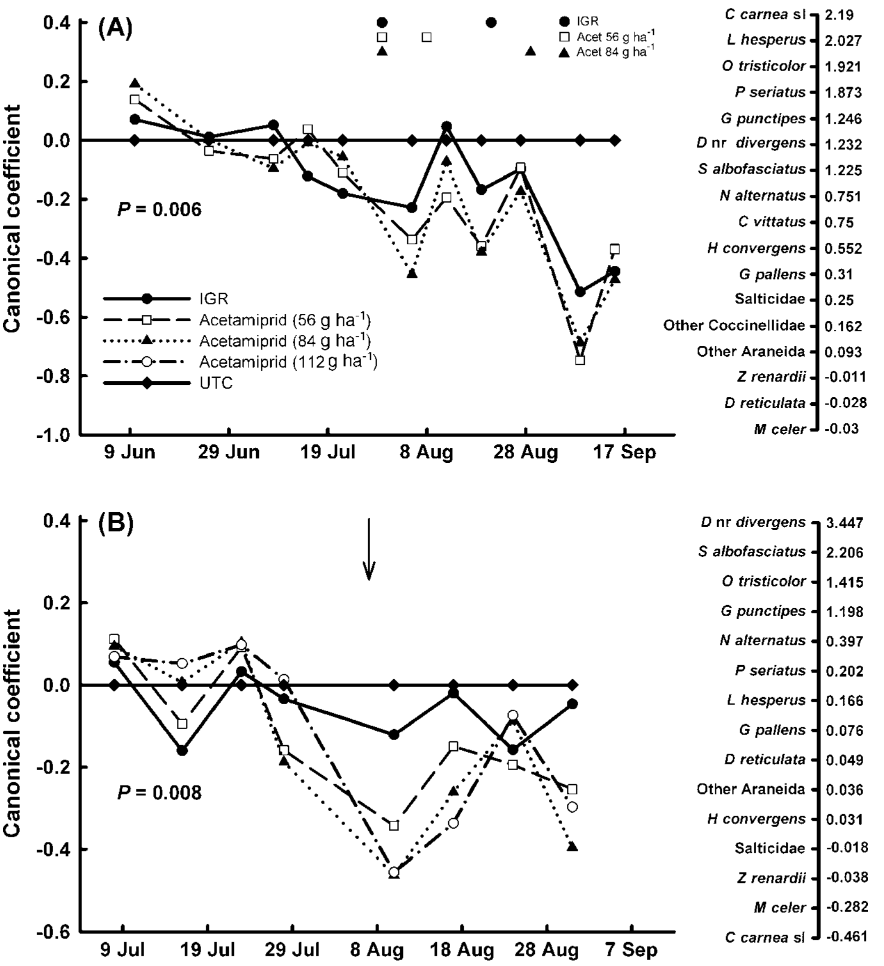

Figure 3. Principal response curves (PRC) showing the effects of different strategies for control of *Bemisia tabaci* on the predatory arthropod community in (A) 1997 and (B) 1998, Maricopa, Arizona, USA. The PRC show the effect of each treatment regime relative to the untreated control which is represented by the $y = 0$ line. The greater the species weight the more the response for that species resembles the PRC; negative weights indicate an opposite pattern. The product of the species weight and the canonical coefficient for a given treatment and time equals the natural log change in density of that species relative to the control. The *P* value denotes the significance of the PRC analysis over all dates based on an *F*-type permutation test. Symbols alone along the top of graph A denote the timing of insecticide applications in 1997; the arrow above graph B denotes the timing of a single application in all treatments in 1998. Insecticide rates given as grams of active ingredient per hectare.

systems have become available. These evolving strategies have also progressively fostered the increasing role of other tactics, such as cultural control, area-wide implementation and conservation biological control, as components of robust IPM.[14,16,43] Current recommendations for the management of *B tabaci* on cotton in Arizona and California call for routine field monitoring and adherence to action thresholds so that insecticides are used only when needed. When insecticides are needed it is recommended that the IGRs buprofezin or pyriproxyfen be utilized first in preference to other available materials.[14] These materials are highly effective and have the further advantages of novel modes of action and selectivity that conserves natural enemy populations early in the crop cycle, permitting biological control to contribute significant mortality to whitefly and other pest populations.[43] However, new insecticides continue to be introduced and changing economics, shifts in grower practices, and the ever-present issue of reduced susceptibility to current insecticides by *B tabaci* requires that new potential management scenarios be tested and evaluated.

Acetamiprid is one of several neonicotinoids that have been introduced in the past decade, and our finding of its high efficacy against *B tabaci* is consistent with published studies on cotton and other affected crops.[9,21,44–46] Our findings also support the results from published bioassay data that acetamiprid has activity against various natural enemies. Our results suggest a rather broad taxonomic spectrum of activity including predatory beetles, predaceous and omnivorous bugs, green lacewing and predatory flies. By comparison, an insecticide regime based on the use of various mixtures of pyrethroids, organophosphates, carbamates and cyclodienes significantly affected more predator taxa, including some spiders,[16] but for those taxa affected by both acetamiprid and these conventional insecticides, the negative effects were roughly equal (see Table 4). The IGRs did negatively affect several predator taxa here, and this is consistent with our prior results.[15,16] However, the overall impact of the IGRs was less than that caused by either acetamiprid or mixtures of conventional materials.

Table 4. Meta-analysis of the effect of *Bemisia tabaci* suppression on arthropod predators, predator:prey ratios, and parasitism relative to an untreated control, Maricopa, Arizona, USA[a]

Predator	Acetamiprid		IGR		Conventional[b]	
Dictyna reticulata	0	(0.69)	0	(1.06)	0	(0.41)
Misumenops celer	0	(0.89)	0	(0.84)	−0.50	(0.51)
Jumping spiders	0	(0.75)	0	(0.45)	0	(0.52)
Other spiders	0	(0.47)	0	(0.45)	−0.46	(0.75)
Collops vittatus	−0.76	(0.24)	0	(0.42)	−0.70	(0.30)
Hippodamia convergens	−0.41	(0.22)	0	(0.79)	0	(0.34)
Other coccinellids	0	(0.58)	0	(0.68)	0	(0.92)
Geocoris punctipes	−0.23	(0.48)	0	(0.64)	−0.61	(0.39)
Geocoris pallens	0	(0.53)	0	(1.11)	−0.32	(0.68)
Orius tristicolor	−0.48	(0.52)	0	(0.83)	−0.44	(0.56)
Nabis alternatus	0	(0.27)	0	(1.59)	−0.40	(0.31)
Zelus renardii	0	(0.80)	0	(1.10)	−0.45	(0.47)
Lygus hesperus	−0.21	(0.75)	−0.18	(0.89)	−0.42	(0.58)
Pseudatomoscelis seriatus	−0.39	(0.60)	−0.25	(0.53)	−0.67	(0.33)
Spanogonicus albofasciatus	−0.71	(0.29)	−0.42	(0.46)	−0.38	(0.31)
Chrysoperla carnea sl	−0.36	(0.51)	−0.34	(0.65)	−0.31	(0.70)
Drapetis nr *divergens*	−0.27	(0.64)	0	(0.78)	−0.45	(0.55)
Pred:prey ratio	0.81	(0.81)	1.37	(1.37)	0	(1.15)
Proportional parasitism	−0.33	(0.60)	−0.40	(0.60)	0	(0.83)

[a] Index is calculated as the mean of $p_i s_i$ over both years, where p is the proportional change in predator density, parasitism, or the predator:prey ratio in a given insecticide regime relative to the control in year i and s is a dummy variable indicating the statistical significance ($s = 1$) or non-significance ($s = 0$) of the reduction based on ANOVA results in year i. Values in parentheses indicate the mean (both years) density, ratio or parasitism rate as a proportion of the control level irrespective of statistical significance.

[b] For reference, results from a concurrent study using a rotation of conventional materials (pyrethroids, organophosphates, carbamates, cyclodienes) conducted at the same research center in 1997 and 1998.[16]

Field studies of insecticide effect on natural enemies integrate many factors, including direct toxicological effects and indirect effects such as reductions in prey availability. Direct toxicological effects of acetamiprid have been studied using laboratory and field residue bioassays for several natural enemies. Mori and Gotoh[27] found acetamiprid toxic to *Scolothrips takahashi* Priesner, a predatory thrips, and the coccinellid beetle *Stethorus japonicus* H Kamiya, and Grafton-Cardwell and Gu[23] reported reduced larval and adult survival as well as reduced reproduction in the coccinellid *Rodolia cardinalis* (Mulsant). Acetamiprid was also found toxic to most of the developmental stages of the coccinellid *Harmonia axyridis* (Pallas).[29] Coccinellids were relatively rare at our study site, but *H convergens* was negatively affected by acetamiprid and at a level more severe than that by mixtures of conventional insecticides. Acetamiprid was found highly toxic to adults of the aphelinid parasitoid *Encarsia formosa* Gahan, and four predatory bugs, *Macrolophus caliginosus* Wagner, *Orius laevigatus* (Fieber), *O majusculus* (Reuter) and *O insidiosus* (Say);[26,28] however, Viggiani *et al*[30] reported that larval *E formosa* using greenhouse whitefly as a host were not affected. Acetamiprid had relatively little effect on parasitism by aphelinid parasitoids here, but overall rates of parasitism were very low in both years. We did, however, see negative effects by acetamiprid on several predaceous and omnivorous bugs, most notably *O tristicolor*. This was somewhat predictable from the systemic translaminar activity of acetamiprid and other compounds in this class and from the plant-feeding behavior demonstrated by many predaceous bugs.[47] Although this systemic activity would presumably minimize residual contact on the undersurface of a leaf treated from above, predators that commonly feed on plant sap would have increased exposure to the toxicant regardless of where they foraged and fed. A final study reported minor effects from various exposures of *C carnea* to acetamiprid,[31] whereas we found that acetamiprid significantly reduced populations of this predator by an average of about 36%.

In addition to potential direct toxicological effects, the high efficacy of both acetamiprid and the IGRs led to large reductions in density of the most abundant prey that may have indirectly affected predator population dynamics. The gradual decline in predator populations in the IGR regimes relative to the untreated control over the season (see Fig 3) was coincident with a similar decline in densities of whitefly prey. In contrast, more immediate and severe reductions in predator populations followed applications of acetamiprid. This exact pattern was observed with the use of IGRs in comparison with mixtures of conventional insecticides in a prior study.[16] Predator:prey ratios based on the entire predator complex further highlight the differential effects of prey density and direct toxicity between the IGRs and acetamiprid. The IGR regime consistently resulted in higher predator:prey ratios than acetamiprid regimes. Predator:prey ratios based more specifically on the impact of each taxon would likely be more meaningful; however, such knowledge is currently lacking. Presently our understanding of quantitative rates of predation on *B tabaci* is based on the collective activity of the entire predator complex.[43] None the less, all else being equal, higher overall ratios would be more favorable to biological control. The tangible effects of insecticide selectivity and consequent natural enemy conservation have been demonstrated through life table studies which show that predation contributes significantly to season-long suppression of *B tabaci*[43] (Naranjo SE and Ellsworth PC, unpublished).

The polyphagous nature of *B tabaci* has forced explicit recognition of the fact that management of this pest on any one crop is inexorably tied to others in the same region. Not only is the pest shared among crops, but so are the insecticides used in its control. Since 1998, researchers in Arizona, in cooperation with the agrichemical and crop production industries, have been developing strategies for insecticide resistance management that apply to all affected commodities simultaneously.[9,14] In part, this was prompted by the expanded registration of buprofezin to crops other than cotton, and more recently the expanding registration and use of several neonicotinoids on multiple crops. The current cotton plan[48] continues to support the three-stage system discussed, which calls for the initial usage of selective IGRs. The second stage now includes the use of neonicotinoids, including acetamiprid, in areas of the state where cotton is the dominant crop or where cotton and melon production overlap, but eliminates their use in areas with a large vegetable production area, so as to preserve the efficacy of these materials for future vegetable crop protection. Our results are consistent with the continued philosophy that the IGRs represent the only selective materials currently available for *B tabaci* control in cotton and should be used preferentially in the initial stage of control for this pest. Although some growers have begun to use acetamiprid in Stage I, it will not provide the same benefits to which producers have become accustomed—a long residual effect due in part to the chemistry but more importantly to the preservation of natural enemies.[14,43] If the industry adheres to the cross-commodity guidelines it is likely that the important activity of IGRs, neonicotinoids and older classes of chemistry will be maintained and that changes to the current cotton management plan will not be needed. However, in the event that changes are needed in the future, acetamiprid would be a poor substitute for the IGRs in the initial stage of control and would likely compromise a successful integrated control program. Consistent with current recommendations, the best fit for acetamiprid would be in Stage II were selectivity is less important.

ACKNOWLEDGEMENTS

We thank T-X Liu (Texas A&M), John Palumbo (University of Arizona), and David Schuster (University of Florida) for helpful comments on earlier drafts of this manuscript, Kim Hoelmer (USDA-ARS, Montpellier, France) for identification of parasitoids and Kim Beimfohr, Barbara Hefner, Stephanie Jones and Gregory Owens for expert technical assistance. Partial funding was provided by the former Rhone-Poulenc Ag Company, Durham, NC, USA.

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