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Field evaluation of different insecticide use strategies as resistance management and control tactics for *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract

Various insecticide use strategies including rotations, sequential use, and mixtures were evaluated experimentally on *Bemisia tabaci* (Gennadius) in California and Arizona (USA) cotton fields. Toxicological responses of adult *B. tabaci* were measured along with preimaginal densities and cotton yields from plots subjected to different insecticide regimens. Weekly monitoring for susceptibility changes over ten consecutive weeks in four different trials failed to detect significant differences between sequential use and rotation regimens, nor in comparison to the control plots. There were, however, significant differences among study-site locations and between study years as well as significant within-season time effects. Relative infestations in insecticide-treated plots expressed as a percentage of preimaginal densities in control plots indicated that better control was obtained by all insecticide treatments in conjunction with higher susceptibility levels observed in the second year. Lower preimaginal densities of *B. tabaci* were measured in the rotation treatment in comparison to sequential treatments of endosulfan, chlorpyrifos, or amitraz, but all were less effective than sequential treatments of bifenthrin or the mixture of bifenthrin + endosulfan. Cotton lint yields were inversely related to *B. tabaci* densities, with highest yields in the bifenthrin and mixture plots and lowest yields in the control plots. Suppression of *B. tabaci* infestations in insecticide-treated plots relative to untreated control plots also improved under conditions of lower *B. tabaci* pressure. The increases in cotton yield and susceptibility to insecticides seen in the current study support the trend observed in the southwestern USA of improved management of *B. tabaci* despite continuing intensive use of insecticides.

Introduction

The evolution of pesticide resistance in insect pests is a complex evolutionary process (Rosenheim & Tabashnik, 1990) that is dependent on a number of biological, genetic and/or ecological factors (Georghiou & Taylor, 1977a,b). The relative importance of these factors to the development of resistance can vary in different ecological environments. Depending upon the circumstances, certain insecticide-use strategies may be more effective than others at delaying the onset of insecticide resistance in a particular pest and geographical area. Among the more common strategies that have been characterized include the use of insecticides

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sequentially, in mixtures or in rotation (Georghiou, 1983; Curtis, 1985; Tabashnik, 1989). Georghiou (1994) further defined resistance management tactics according to the intensity of insecticide exposure (moderation vs. saturation) and the sequence and/or diversity of insecticides (multiple attack) that are applied. The particular strategy employed ideally should account for the risks of resistance developing to the candidate insecticide(s) based on knowledge of the biology and ecology of the pest species (Keiding, 1986; Georghiou, 1994). Even in the absence of detailed knowledge, however, Roush (1989) encouraged field entomologists to institute a 'first approximation' resistance management programme, choosing from a limited set of basic options at first, and then fine tuning as more information becomes available with experience.

Examples of insecticide resistance management programmes that have been implemented on a production basis remain relatively limited in view of the considerable attention devoted to formulating strategies designed to combat resistance (Roush, 1993, 1989; Tabashnik, 1990; Forrester, 1990). Results have been favourable for the handful of insecticide resistance management (IRM) programmes being practiced, especially when contrasted to similar systems with the same insect pest(s), but with no IRM in place (Forrester *et al*., 1993; Horowitz *et al*., 1994). However, the gap between theory and practice (Denholm & Rowland, 1992) remains substantial, in part because too few programmes have been implemented, but also because there has been too little empirical testing of theoretical ideas. Much of the evaluation to date has been in the form of computer simulations (Rosenheim & Tabashnik, 1990; Gould, 1991; Caprio & Tabashnik, 1992; Goss & McKenzie, 1996). These provide valuable insight into the frequencies of resistance genes within populations under varying conditions, although they are often constructed from a set of simplified and often untested assumptions. Nonetheless, computer simulations have permitted testing of competing resistance management strategies in an effort to forecast the approach that yields the lowest resistance for the longest period, and by doing so have fortified a theoretical base from which empirical data may be compared.

Efforts to measure resistance changes in fields experimentally subjected to various insecticide regimens have been notably few. Immaraju *et al*. (1990) looked at responses of populations of citrus thrips, *Scirtothrips citri* (Moulton) (Thysanoptera: Thripidae), exposed to insecticide use strategies that consisted of sequential (continuous) or rotational use of insecticides, or as mixtures, in different citrus groves in the San Joaquin Valley, California. They found that resistance levels increased most under the sequential regimen and that rotation of insecticides was superior to mixtures. McKenzie & Byford (1993) examined these same insecticide-use strategies with respect to patterns of insecticide resistance development in horn flies, *Haematobia irritans* (Linnaeus) (Diptera: Muscidae), albeit under more artificial conditions in that steer hosts of the horn flies were maintained in environmentally controlled rooms. The highest resistance level they observed developed in the single insecticide, sequential use scheme, whereas treatments with mixtures or rotations of insecticides yielded much lower resistance. Similarly, MacDonald *et al*. (1983a) studied responses of houseflies, *Musca domestica* (Linnaeus) (Diptera: Muscidae), in the laboratory under different spray regimens and concluded that, of all strategies, mixtures best avoided resistance. However, the same strategies applied to houseflies on farms under normal production circumstances produced variable results (MacDonald *et al*., 1983b).

During the last two decades, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) has become an increasingly serious pest of agriculture and more recently of floriculture (Osborne, 1988; Parrella *et al*., 1992). The intensive reliance on insecticides in field and vegetables crops and protected agriculture renders *B. tabaci* a strong candidate for the selection of resistance to insecticides. Resistance to various insecticides belonging to different classes has been well documented in *B. tabaci* from around the world (Dittrich *et al*., 1985, 1990; Prabhaker *et al*., 1985, 1992; Horowitz *et al*., 1988; Cahill *et al*., 1995) and has been implicated as a factor in its elevated pest status (Dittrich *et al*., 1985). Differences among populations of *B. tabaci* in host range, protein and nucleic acid patterns, and mating compatibility has brought recognition of different biotypes or 'strains' at the very least, and perhaps different species (Perring *et al*., 1993; Bellows *et al*., 1994). The pattern with respect to insecticide resistance development remains consistent, however, irrespective of strain or species (Cahill *et al*., 1995).

As concerns for development of resistance in *B. tabaci* to the various insecticides increase due to their intensive use for control, it is essential to develop practical insecticide resistance management (IRM) strategies that will potentially delay the onset of resistance to insecticides. With the widespread destruction across the southern USA in the past 10 years due to *B. tabaci* outbreaks, and given its history of insecticide resistance, there was a pressing need for research concerned with empirically testing different resistance management strategies towards the eventual implementation of an insecticide resistance management (IRM) programme. Having observed that laboratory populations of *B. tabaci* developed resistance to a lesser degree and slower rate when subjected to different resistance management approaches compared to consistent treatment with a single insecticide (Prabhaker *et al*., 1998), the question arose as to how natural populations in the field would respond to these insecticide resistance management approaches. Our objective was to apply the same rotation and mixture strategies used in the laboratory selection and evaluate them relative to a conventional insecticide use strategy that involved the sequential, or continuous use of one compound through a crop season by measuring toxicological responses of *B. tabaci*. The same insecticides that were used in the laboratory study were applied in the field evaluation, but using field rates. Additionally, towards the goal of establishing a viable resistance and pest management programme for *B. tabaci*, we were also interested in exploring the control efficacies of different insecticide use strategies by measuring whitefly densities and cotton yields in field plots subjected to different insecticide use regimens, i.e. to evaluate the pest management potential of these strategies.

Materials and methods

Study sites

Trials were conducted simultaneously at two locations each year in 1994–95. The experimental crop for all four trials was cotton, selected for its consistent exposure to heavy populations of *B. tabaci* during the summer months in

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the desert southwest. Both trial locations in 1994 were in the Imperial Valley, California. One was at the University of California Desert Research and Extension Center near Holtville, and the second was at the USDA Irrigated Desert Research Station near Brawley. The Holtville site was used again in 1995, but the second site was at the University of Arizona Agricultural Center in Yuma, Arizona to take advantage of lower *B. tabaci* immigration pressure than that experienced in the Imperial Valley during the previous year. This is a distance of about 80 km from Holtville, and the distance between Holtville and Brawley is about 30 km.

Field planting and layout

Preplant field preparation was identical at all four sites, involving disking, land planing, and application of nitrogen and phosphate fertilizers in the form of urea (36–0-0) and ammonium phosphate (11–52–0). Fields were planted with Deltapine cultivar '5261' cotton and first watered 5–15 March each year. Four to five weeks after germination, plants were thinned to 10–15 cm spacing and furrow irrigated every 2–4 weeks. At the squaring stage in June, irrigation was set on a weekly schedule to accommodate the tractor-driven sprayer for applying treatments, but still provide sufficient water under very high temperature conditions. Spraying commenced in late June or early July depending on whitefly densities in the experimental plots.

Treatment plots for each trial were laid out within one contiguous block. The overall area of the blocks varied slightly at the different sites, but all were between 1.8 and 2.0 ha. Identical plot dimensions were used at each site without any untreated buffer rows between plots. The same seven treatments were tested at all four sites, with two replicates per treatment that required a total of 14 plots per site. Plot dimensions were 16 rows by 30 m with 1 m row spacing.

Insecticides

The following formulations were used: bifenthrin (Capture® 2 EC) and endosulfan (Thiodan® 3 EC), both supplied by FMC Corp., Princeton, New Jersey; chlorpyrifos (Lorsban® 4 E) from DowElanco, Indianapolis, Indiana; and amitraz (Ovasyn® 1.5) from AgrEvo, Wilmington, Delaware. The following rates (kg (ai) ha^{-1}) were applied in both the sequential and rotational treatments: bifenthrin (0.112), endosulfan (1.12), chlorpyrifos (1.12) and amitraz (0.28). For the mixture treatment, the rate for bifenthrin was 0.056 kg (ai) ha^{-1} and for endosulfan 0.56 kg (ai) ha^{-1}.

Insecticide applications

Insecticide treatments used in the present study were selected to represent insecticide classes with different modes of action. Treatments 1–4 (and their respective insecticide classes) were continuous applications of bifenthrin (pyrethroid), endosulfan (cyclodiene), chlorpyrifos (organophosphate) or amitraz (formamidine). The repeated use of these four insecticides within their respective plots represented a sequential insecticide use strategy, so called because of the pattern observed in chemical management of using an insecticide constantly until it no longer provides adequate control, then moving on to another product in sequence. Treatment 5 was a rotation strategy that used the same four insecticides, but on a rotational basis applied over two cycles beginning with bifenthrin and followed in order by endosulfan, chlorpyrifos and amitraz. The sixth treatment was a mixture of bifenthrin + endosulfan that was applied weekly. All six treatments plus an untreated control were replicated twice at each study site (table 1).

Insecticide applications at both sites in 1994 commenced the week of 13–17 June and continued for eight consecutive weeks, allowing for two rotation cycles to be completed. In 1995, spraying at the Holtville site began the week of 19 June, but in Yuma, *B. tabaci* densities were too low to begin at this time. Consequently, it was not until the week of 17 July that treatments began in Yuma. Treatments at both sites continued for eight weeks, terminating the week of 21 August in Holtville and 18 September in Yuma.

The application rates of the insecticides in their respective treatments were constant for all four trials. The rates used in the various treatments generally matched the commonly used rates in commercial cotton fields. Commercial grade insecticides were used for field applications as well as for bioassays. All treatments were applied with a high-pressure hydraulic sprayer (Specialty Agricultural Equipment, Reedley, California) at the rate of 187 l ha^{-1} at 27 kg cm^{-2}. Four rows were sprayed simultaneously using three fanjet nozzles per row. Spraying began when adult whitefly numbers reached a level where 60% of fifth mainstem-node leaves were infested with two or

Table 1. Resistance management strategies tested in the field in 1994–95, insecticides, and their rates of application over eight consecutive weeks at all four field sites.

Resistance management strategy	Insecticides used	Application regimen	Rates (kg (ai) ha^{-1})
Sequential	Bifenthrin	Each insecticide sprayed	0.112
	Endosulfan	weekly for 8 consecutive	1.12
	Chlorpyrifos	weeks in its respective	1.12
	Amitraz	plots	0.28
Rotational	Bifenthrin	Rotated in the order	0.112
	Endosulfan	shown (top to bottom)	1.12
	Chlorpyrifos	for two cycles over	1.12
	Amitraz	the 8 week period	0.28
Mixture	Bifenthrin + endosulfan	Sprayed weekly for	0.067
		8 consecutive weeks	0.56

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more adults, and continued weekly through the period of highest *B. tabaci* pressure.

Bioassay technique

A yellow sticky card technique described by Prabhaker *et al*. (1992, 1996) was used to monitor weekly responses of *B. tabaci* populations to the respective insecticides for ten consecutive weeks. This method involved spraying serial dilutions of the insecticides with a Potter Spray Tower onto 7.5 × 12.5 cm plastic yellow cards to which an adhesive (Tanglefoot, Michigan) had been applied. A set of five cards was prepared for each insecticide, and each card in the set was treated with a specific concentration. The series of concentrations on individual cards was selected to produce a range of mortalities between 5 and 95%. Treated cards were then carried to the fields in ice chests and exposed to *B. tabaci* adults from each plot until 60–100 subjects were caught in a 3.8 cm^2 grid printed in the centre of the card. Exposure times varied between 15 and 60 s, with each card returned to its ice chest immediately after catching sufficient numbers. After loading all cards in the field, they were returned to the laboratory and transferred to a 0.022 m^3 ice chest containing 4 l of water and incubated for 24 h. The incubation chests were covered and maintained at room temperature and in high humidity provided by the enclosed water. Mortality was determined under a dissecting microscope by checking for lack of movement in the immobilized *B. tabaci* adults when prodded.

Duplicate sets of yellow sticky cards were prepared for all 14 plots (6 treatments + untreated control = 7 × 2 replicates) at each field site. With the four sequential treatments, yellow card sets were prepared to match the insecticide applied to the plot, e.g. bifenthrin-treated cards to measure responses of *B. tabaci* collected in bifenthrin-treated plots. For the rotation plots, sets of cards for all four insecticides used in the rotation schedule were prepared each week for the bioassays. *Bemisia tabaci* adults in the untreated control plots were also bioassayed with all four insecticides. Yellow sticky cards treated with the mixture of bifenthrin + endosulfan were used to test *B. tabaci* responses in the mixture treated plots, but also in the rotation and control plots.

All sets of bioassay cards were prepared early mornings on the same day that *B. tabaci* adults were collected. Sampling at the field sites was done seven days after the previous spray application and just prior to the next application. On the following day, all bioassay sets were read and mortalities at each concentration recorded. The process was repeated beginning the following day, but this time in preparation for the second field site. This schedule was maintained through the eight week application period and ten week monitoring period.

Insect densities

Field evaluation of the relative control provided by the different insecticide regimens was conducted in tandem with the resistance management study. To estimate relative densities of *B. tabaci* within each treatment, ten plants per replicate (20 per treatment) were sampled each week prior to application of insecticides. Leaf disks (2.5 cm^2) were punched from the proximal portion of fifth mainstem-node leaves using a precision sampler punch (Birkestrand Co.) and collected into an attached jar. Each set of ten disks was transferred into a labelled vial to which 70% ethanol was added for storage. Leaf disks were removed from the ethanol and assessed individually under a dissecting microscope for numbers of eggs and nymphs.

Cotton yield

Following the final application of insecticide treatments, study fields were defoliated with thidiazuron (Dropp®, AgrEvo, USA). Two weeks after defoliation, three 5-m sections of cotton row within each treatment replicate were randomly selected for cotton harvest. All cotton within each delineated section was handpicked and placed in individual paper bags. The harvested cotton was ginned and lint cotton weights measured.

Data analyses

All bioassay data were entered as replicated sets for probit analysis using POLO (LeOra Software, 1987). The LC_{50}s and 95% confidence limits were extracted for further analysis provided that the *g* statistic (index of significance for potency estimation) was less than 0.5 (Robertson & Preisler, 1991). Comparisons of LC_{50}s from bioassays on *B. tabaci* adults collected from the sequential, rotation and control plots were made on the basis of whether or not their 95% confidence intervals (C.I.s) overlapped. There were three possible comparisons (sequential vs. rotation or control; rotation vs. control) for each insecticide treatment each week and a possible total of 30 (3 × 10 weeks) for the season at each study site. A weekly composite LC_{50} for each insecticide treatment was produced from the combined data of the sequential, rotation and control bioassays if their 95% C.I.s overlapped. The composite LC_{50}s were used to express the seasonal trend providing that the number of significant differences (non-overlap of 95% C.I.s) among the three comparisons summed within a trial-site season did not exceed a type I error rate of $\alpha = 0.05$. The α level was adjusted using a sequential Bonnferoni correction (Rice, 1990) to account for the large number of comparisons (maximum of 30) within each season.

A univariate repeated measures analysis of variance was conducted using JMP v. 3.0 (SAS Institute, 1994) to identify sources of significant variation in the responses of *B. tabaci* over the four trials. Insecticide use strategy (sequential, rotation, or control) represented the whole plot effect with trial site and year nested within strategy. The time effect was represented by week (wk) and the interaction term of strategy*wk. The residual error term was used to calculate F-values for all effects except for strategy, which used the product of the effects site(strategy) and year(strategy) as the appropriate error term. Log transformed LC_{50}s were used as the response variable.

Egg and nymphal counts were combined and log transformed for analysis of variance. Each week's data was analysed separately within each of the four field trials using a one-way ANOVA for treatment effect. All pairs of treatment means were compared and separated ($\alpha = 0.05$) by the Tukey-Kramer HSD procedure. The number of significant differences each week among treatment pairs was totalled by treatment across all weeks within each trial as a measure of season-long performance. End of season cotton yields were analysed by the same ANOVA and mean separation procedure. All analyses were completed using JMP v. 3.0 (SAS Institute, 1994).

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Results

Toxicological responses of B. tabaci

There were few significant differences among responses of *B. tabaci* collected in sequential, rotation or control plots to any of the five insecticide treatments used in the bioassays. This is illustrated by the complete bioassay data from all three treatment strategies (including control) for bifenthrin (fig. 1). Only one comparison in each of the first three trials represented a significant difference between LC_{50}s, whereas three of the total of 30 comparisons made during the Yuma 1995 season were significantly different. But there was no consistency as to which treatment strategy produced a significantly lower LC_{50} nor any other trend in the data to suggest that the pattern of insecticide use or non-use, i.e. unsprayed control, produced different bioassay responses in whiteflies as evaluated by LC_{50}s. Similarly, for each of the other insecticide treatments tested in yellow sticky card bioassays, the number of significant differences between insecticide-use strategies within a trial-site season did not exceed their respective type I error rates following a Bonnferoni correction (table 2).

The failure of any insecticide-use strategy to produce a significant effect was further elucidated in the ANOVA results (table 3). Insignificant F-values were produced for the strategy term for all five insecticide treatments. However, other terms expressed in the ANOVA model were significant or highly significant. In particular, the term year[strategy] produced highly significant differences for four of the five insecticide treatments tested in the bioassays. This difference between years is visibly acute in a graphical representation of the composite LC_{50}s (+ 95% C.I.s) for bifenthrin, endosulfan, bifenthrin + endosulfan, and amitraz (fig. 2). Responses of whiteflies to these insecticides in the yellow sticky card bioassays produced consistently lower LC_{50}s in 1995 compared to 1994. The sites at which these field studies

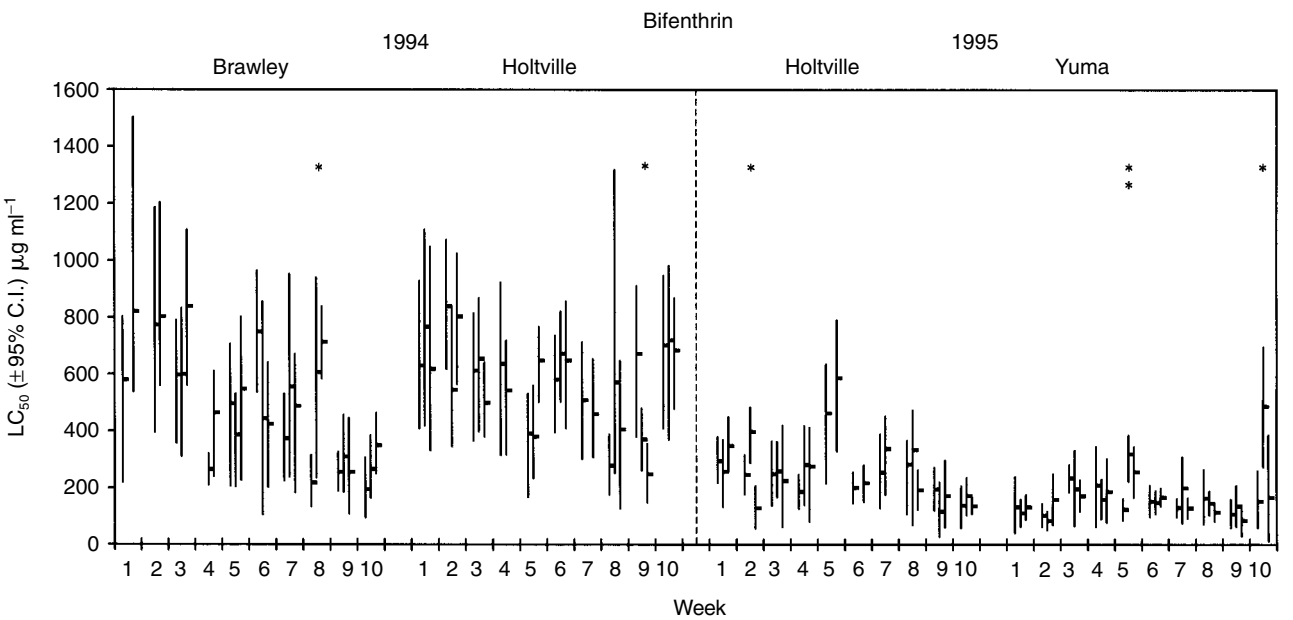

Fig. 1. Comparison of bioassay results for bifenthrin obtained with *Bemisia tabaci* collected from sequential, rotation or control plots. Mortality responses are presented as LC_{50}s (−) with their 95% confidence intervals (|). A series of three (but sometimes only two) LC_{50}s (+ 95% C.I.s) representing (from left to right) the rotation, sequential and control treatment strategies are given for each week at the four different trial sites.

Table 2. The number of significant differences among LC_{50}s[a] (represented by the numerator and based on non-overlap of 95% confidence intervals) from bioassays of adult *Bemisia tabaci* collected in sequential, rotational, or control plots out of the total number of comparisons (denominator; maximum of 30) per trial-site season.

Trial site and year	Bioassay treatment				
	Bifenthrin	Endosulfan	Bifenthrin + endosulfan	Chlorpyrifos	Amitraz
Brawley, 1994	1/24	0/26	1/30	3/26	2/28
Holtville, 1994	1/26	2/28	0/30	2/28	0/24
Holtville, 1995	1/24	1/30	0/28	1/18	0/27
Yuma, 1995	3/30	2/22	1/24	2/23	1/22

[a] Only LC_{50}s with a g value ≤ 0.5 were used for comparisons.

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Table 3. Summary of a repeated measures analysis of variance for bioassay data using log-transformed LC_{50}s as the response variable.

Source of variation	Bioassay treatment				
	Bifenthrin	Endosulfan	Bifenthrin + endosulfan	Chlorpyrifos	Amitraz
Strategy $_{(2, 3.9)}$[a]	0.01	0.01	0.02	0.16	0.10
Site[Strategy] $_{(6,81)}$	4.47***	2.23*	4.73***	2.10	2.12
Yr[Strategy] $_{(3,81)}$	23.28***	29.92***	22.22***	1.72	17.86***
Wk $_{(9,81)}$	3.03**	3.16**	4.74***	0.91	2.94**
Strategy*Wk $_{(18,81)}$	0.64	0.25	0.33	0.28	0.56

[a]Degrees of freedom (numerator, denominator) for each source of variation; a synthetic denominator for the strategy term. These were the same for all treatments except amitraz which had a denominator degrees of freedom of 74.
F-values for each insecticide treatment are presented with significant values notated (*, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$). Analyses were performed separately for each insecticide treatment

were carried out, represented by site[strategy], also had a significant effect on the responses of the locally sampled *B. tabaci* when tested with bifenthrin, endosulfan or a mixture of these (table 3). This model effect was nearly significant ($F_{(6,81)} = 2.10$, $P = 0.06$) for chlorpyrifos as well, but at least the trend of lower LC_{50}s in 1995 compared to the previous year was consistent with the other treatments (fig. 2).

There were significant time effects represented by the term week within each trial season that were observed for all insecticide bioassays except chlorpyrifos (table 3). In some instances, a tendency towards lower LC_{50}s at the end of the 10-week monitoring period was observed. This is evident for bifenthrin, endosulfan and amitraz at the Brawley site in 1994, and for amitraz at Holtville in 1994 (fig. 2). There was no significant interaction between time and insecticide use strategy (table 3).

Bioassay responses of *B. tabaci* adults to the mixture of bifenthrin + endosulfan ranged from 8 to 30 times more sensitive (average of 15×) than when bifenthrin was tested singly. Similarly, an increase in mortality was observed when endosulfan was used in combination with bifenthrin, but only 1.2–8 times greater (average of 2.6×) than when used alone. Thus, the higher toxicity of the bifenthrin + endosulfan mixture apparently was due to the enhanced activity of bifenthrin. Superior control of *B. tabaci* in field trials through the use of pyrethroid mixtures corroborate the higher toxicities observed in bioassays.

Bemisia tabaci *densities*

Regional pressure differed greatly between the Imperial Valley sites and the Yuma Valley site. This difference was most evident by comparing densities of preimaginal *B. tabaci* in control plots (fig. 3). Increases in the number of eggs and nymphs began in early June at Brawley and Holtville in 1994, but occurred about two weeks later at Holtville in 1995. By mid-July, however, immature *B. tabaci* densities at the 1995 Holtville site equalled those of the previous year, eventually eclipsing the 1994 control densities in late season (fig. 3). In marked contrast to these data is the profile of control densities in Yuma in 1995. Immigration into the Yuma cotton plots was very gradual and late relative to the Imperial Valley field plots. It was not unti September that *B. tabaci* densities reached a moderate level relative to the control plot densities observed at the three trials in the Imperial Valley (fig. 3).

Similar to the improvement in *B. tabaci* susceptibility to insecticides from 1994 to 1995, better control of *B. tabaci* was attained with the respective treatment regimens and individual insecticides in 1995. The greater susceptibility to insecticides in 1995 (fig. 2) possibly contributed to the better control observed at both Holtville and Yuma sites relative to the 1994 trials (fig. 4). These comparisons also demonstrated the overall greater control provided by the bifenthrin and mixture treatments across all four trials. The rotation regimen generally performed better than the sequential treatments of amitraz, chlorpyrifos or endosulfan, and in 1995 approached the level of control observed for the bifenthrin and mixture treatments (fig. 4).

A summary of statistically significant differences among treatments for each of the four field trials (fig. 5) more clearly identified superior treatments, i.e. those with the lowest densities of *B. tabaci* eggs and nymphs. The continuous use of bifenthrin in the sequential treatments was the only treatment that was never statistically inferior to any other as indicated by only positive (significantly lower densities) comparisons to other treatments in all four field trials (fig. 5). The mixture of bifenthrin + endosulfan was nearly as consistent as bifenthrin with the exception of one negative comparison (significantly higher densities) in the 1994 Brawley trial (fig. 5). However, the mixture treatment had the greatest number of positive comparisons to all other treatments at both the Holtville and Yuma sites in 1995. The rotation treatment had an equal number of positive comparisons as the mixture treatment at the Brawley 1994 field, but was not as effective at suppressing *B. tabaci* as the bifenthrin or mixture treatment at the other three field trials (fig. 5). For the chlorpyrifos and amitraz sequential treatments, a higher proportion of significant comparisons for all four field trials were negative, whereas the endosulfan treatment was split about evenly between positive and negative comparisons (fig. 5).

Cotton yield

Differences among treatments in cotton yields reflected the level of *B. tabaci* control provided by the respective treatments as well as the intensity of whitefly pressure on the various trials. The highest yielding trial overall was at

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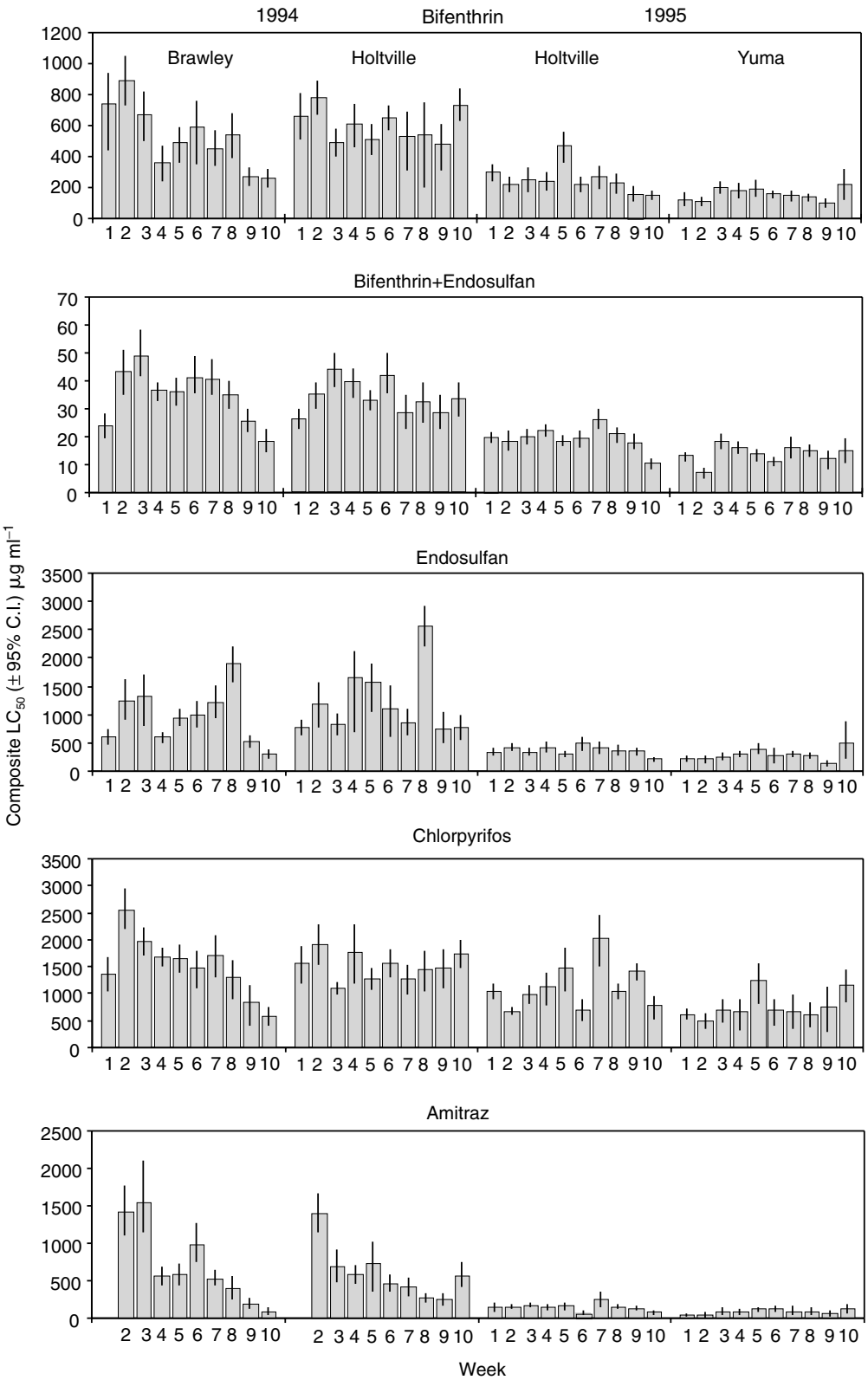

Fig. 2. The composite mean LC_{50}s (+ 95% C.I.s) produced from the combined rotation, sequential and control bioassay data for each insecticide treatment. The number of insects represented by each composite LC_{50} ranged from 1559 to 2648. Bioassays were conducted for 10 consecutive weeks at all four trial sites.

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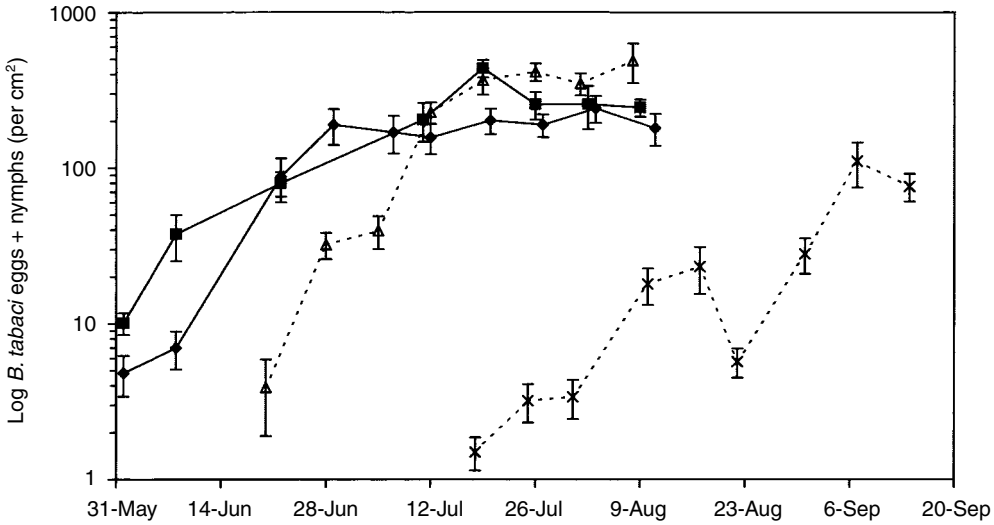

Fig. 3. Relative intensity of *Bemisia tabaci* infestations at the four trial sites (—◆—, Brawley 1994; —■—, Holtville 1994; ··▲··, Holtville 1995; ··✕··, Yuma 1995) according to preimaginal whitefly densities (mean + SEM) in the control plots.

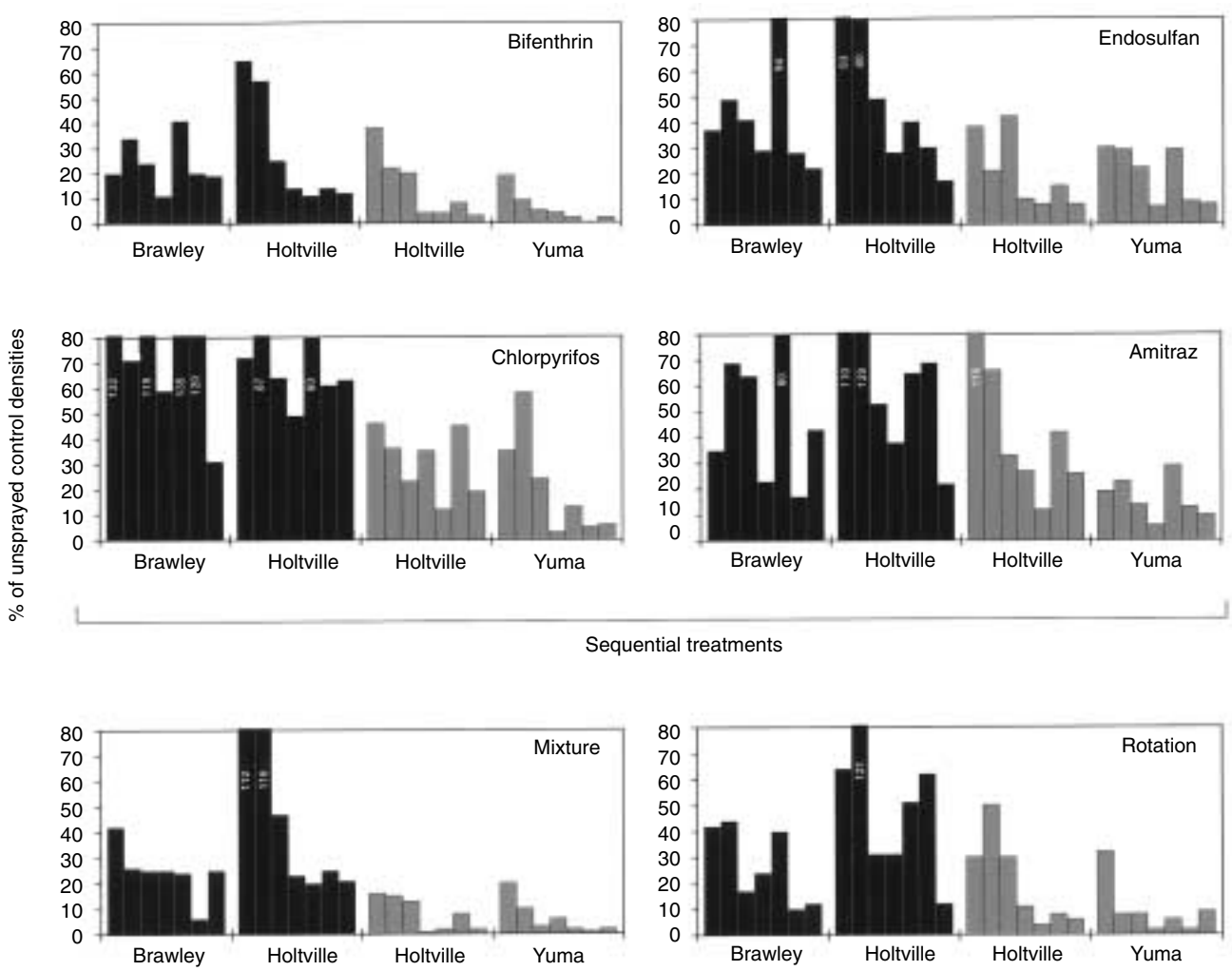

Fig. 4. Weekly densities of preimaginal *Bemisia tabaci* represented as a percentage of the respective weekly densities in unsprayed control plots. The scale (0–80%) is identical for all axes. Numbers at the top of some bars indicate the true percentage of control beyond the 80% limit (sometimes in excess of unsprayed control densities, i.e. >100%). ■, 1994; ■, 1995.

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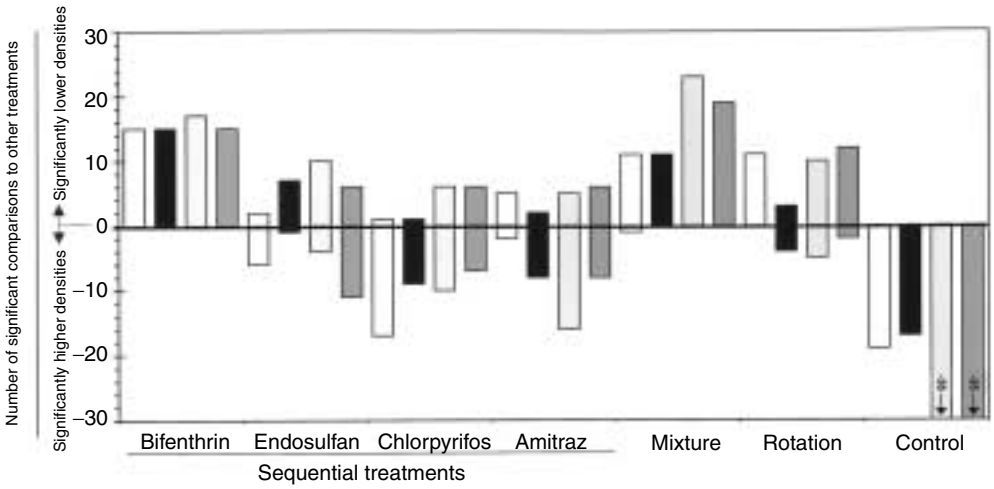

Fig. 5. Whole-season summary of post-hoc comparisons among treatment means following ANOVAs performed on weekly preimaginal *Bemisia tabaci* densities. The four bars for each insecticide treatment represent the cumulative number of positive and/or negative pairwise comparisons per season for each of the four field trials (□, Brawley 1994; ■, Holtville 1994; □, Holtville 1995; ▩, Yuma 1995. Positive comparisons are treatments with whitefly densities that were significantly lower than another treatment, while negative comparisons are treatments with significantly greater whitefly densities than another treatment.

the Yuma site where all treatments except amitraz yielded significantly higher quantities of cotton lint ($F_{(6, 35)} = 3.7$, $P < 0.006$) than the control (fig. 6). Discrimination among treatments at Yuma was not as great as the other trials, possibly because of the relatively late development of the *B. tabaci* infestation. Thus, even the control produced higher cotton yields at Yuma than any treatment at the other three trial sites with the lone exception of bifenthrin at Brawley 1994 (fig. 6). The most consistently performing treatment at all four trials was bifenthrin, although just slightly greater yields (at Holtville: $F_{(6,35)} = 18.1$, $P < 0.0001$) were attained by the mixture treatment in 1995.

The intensity of the *B. tabaci* assault on the Brawley and Holtville sites in 1994 had a detrimental effect on cotton yields when compared to 1995 yields. The more effective control that was observed at Holtville in 1995 compared to 1994 resulted in higher cotton yields in both control and insecticide treatment plots (fig. 6). The higher yields in 1995 occurred despite *B. tabaci* pressure at Holtville that appeared to be similar to 1994 based on control densities. However, the earlier increase in *B. tabaci* numbers in 1994 during a potentially more vulnerable period in the cotton fruiting cycle may have been a factor in the lower yields.

Discussion

Our field study did not demonstrate significant differences in toxicological responses of *B. tabaci* among the various treatments as projected by certain theoretical models (Curtis, 1985; Tabashnik, 1989). A number of factors can be identified that might have contributed to the lack of any significant differences among sequential or rotation regimens, or unsprayed control. Firstly, the time frame for an experiment of this type was rather short, spanning a period of 8–10 weeks. This would be sufficient time for only 3–4 generations of *B. tabaci* to develop within the variously treated plots. Potential changes in resistant gene frequencies under the different insecticide regimens may not have advanced to the point of detection, or perhaps did not vary substantially among regimens. Secondly, the relatively small plot size compared to commercial acreages may not have provided sufficient isolation of *B. tabaci* exposed to different insecticide regimens. Movement of *B. tabaci* between plots may have masked potential differences in relative susceptibility to the various insecticides as a consequence of the treatment regimen. Thirdly, immigration pressure from sources outside of the experimental plots was intensive during the experiment. A substantial proportion of test individuals within any single bioassay may actually have originated from outside of the treatment plots, leading more to a generic response independent of treatment regimen. Moreover, immigration pressure would act to reduce differential selection pressure as a consequence of treatment regimen (Comins, 1977), and therefore produce a similar response in bioassays, again irrespective of the treatment regimen. In contrast to the above resistance-mitigating field conditions, laboratory populations of *B. tabaci* that were subjected to the same insecticide regimens responded positively to resistance-countering insecticide regimens while developing high resistance to bifenthrin under continuous selection (Prabhaker *et al.*, 1998).

Although findings of the present study did not prove that insecticide rotations strategy was superior to the use of mixtures or vice versa to control *B. tabaci* in an open system in Imperial Valley, results from the intensive series of bioassays for each insecticide treatment nonetheless offered valuable information with respect to the status of *B. tabaci* responses to insecticides. The general increase in susceptibility to all of the treatments included in this study from 1994 to 1995 corroborates results from a resistance-monitoring programme in the Imperial Valley conducted over the same time period (Castle *et al.*, 1996a,b). Both the general monitoring data and the results from the present study showed an increased susceptibility to all four classes of insecticides used in this study. It is tempting to suppose that improved susceptibility resulted in improved control of

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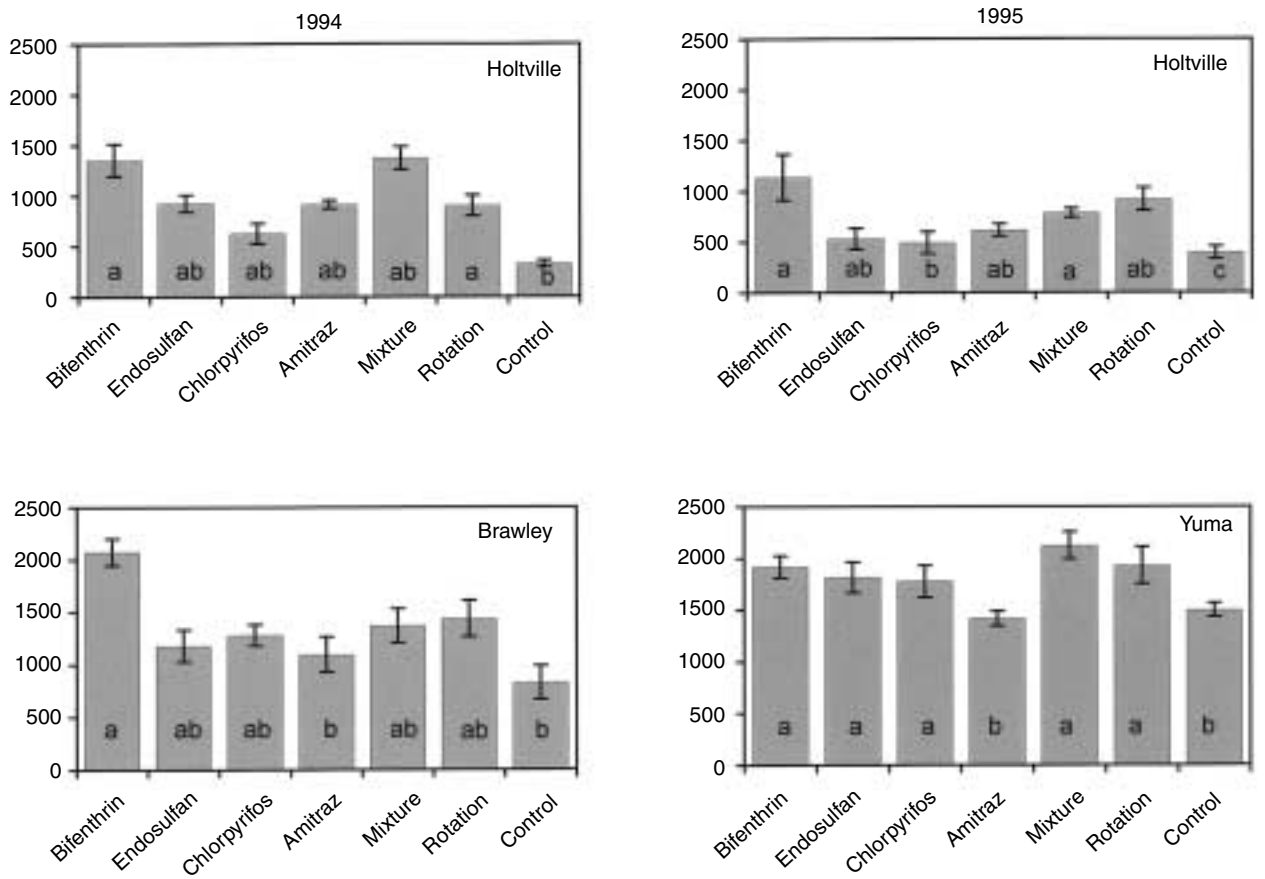

Fig. 6. Mean (± SEM) cotton lint yields for each treatment at each field trial. Bars with unlike letters indicate treatments that differ significantly ($P = 0.05$) following ANOVA and post-hoc comparisons (Tukey's HSD). Scale of vertical axes is identical for all graphs.

whitefly infestations in 1995. But other factors such as lower immigration pressure into the study plots may also have contributed to the lower infestations in 1995. Regional population pressure was much lower at the Yuma site than at the three Imperial Valley sites, although control of *B. tabaci* (as a proportion of unsprayed control densities) at the Yuma 1995 site by the various treatments appeared to be only marginally better than at the Holtville 1995 site.

The increase in susceptibility observed in 1995 to conventional chemistries may in part reflect the impact of exposure in consecutive crops to a new chemical with a novel mode of action on insecticide-resistant *B. tabaci* populations. Following the first year of our study in cotton, imidacloprid was introduced commercially into the Imperial Valley during autumn, 1994 on autumn and winter vegetable crops. It was widely used again on the spring, 1995 melon crop preceding the second year of our field study. The broad-spectrum increase in susceptibility observed in 1995 may have been due partly to a non-selective elimination of resistant and susceptible *B. tabaci* alike during the winter and spring exposure to imidacloprid. There is precedence for resistance-breaking effects that occur upon introducing new chemistry into pest management. Following the introduction of pyrethroids for control of insect pests in Australian cotton in 1978/79, resistance factors to endosulfan dropped from 34.9 during the 1977/78 season to 3.6 the following season, and were further reduced the following three seasons (Forrester *et al*., 1993).

Although the interesting phenomenon of higher rather than lower susceptibility to insecticides was observed during the second year of this study, it was not possible to conclude from a resistance management standpoint which resistance-countering strategy should be practiced for *B. tabaci* in the agricultural valleys of the desert southwest. The neutral results suggest that in certain settings, the impact that localized insecticide use has on the expression of insecticide resistance may be overwhelmed by factors occurring at the regional level. These include the origination of *B. tabaci* on untreated crop and ornamental hosts, exposure to variable insecticide chemistries, and subsequent migration into cotton fields. If these factors are sufficiently expressed, insecticide resistance may not develop as rapidly as has been observed for *Bemisia* spp. in other agricultural settings (Dennehy & Williams, 1997; Denholm *et al*., 1998). This is important not only from the standpoint of assessing resistance risks in particular crops or regions where insecticides are fundamental for protection against *Bemisia* spp., but also for formulating strategies commensurate with the assessed risks.

The general improvement in *B. tabaci* control observed in all treatments in 1995 is supported by the bioassay data that showed increases in susceptibility to all insecticides from

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1994 to 1995. The level of infestation in the respective treatment plots as a proportion of the control decreased substantially in 1995 with a resulting increase in yields. The findings from this study as a whole, i.e. broad-spectrum increases in susceptibility to insecticides and improved control and yields in 1995, indicate that even subtle changes in responses of whitefly populations to insecticides may significantly impact the efficacy of chemical control measures. They also point out that resistance management programmes must be complimentary to the overall pest management concerns, and that region-wide population dynamics may overwhelm locally practiced resistance management tactics.

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