Relative Susceptibility of Whiteflies to Danitol®+ Orthene® Over a 5-Year Period

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Abstract

As part of a program to assess differences in susceptibility to insecticides among regional populations of Bemisia tabaci, insecticide resistance monitoring was carried out at the Maricopa Agricultural Center from fall, 1995 through 1999. Monitoring efforts were concentrated on Danitol®+Orthene® following reports of control problems and documentation of resistance to this mixture in 1995. We were interested in the longer-term dynamics of resistance in light of radically altered treatment regimens beginning with the use of IGRs in 1996. Although the frequency of susceptible individuals to Danitol+Orthene tended to increase in the later years, highly resistant individuals were still present 5 years after the resistance episode of 1995. Whitefly adults collected from various insecticide treatment plots other than Danitol+Orthene were generally uniform in their responses from the time of initial whitefly infestation until defoliation. However, a dramatic shift in susceptibility occurred following a single application of Danitol+Orthene in 1997 and 1999. The increased frequency of resistant individuals following treatment suggests that any large scale return to the use of Danitol+Orthene could rapidly select for proportionally higher numbers of resistant whiteflies and perhaps reduced control in cotton fields.

Introduction

A pivotal point in whitefly management in Arizona cotton was reached in 1995 with the occurrence of intolerable yield losses and lint damage despite heavy use of insecticides that formerly had provided effective control. In particular, the premier chemical treatment of Danitol®+Orthene® proved inadequate to counter late season infestations of *Bemisia tabaci*. During the same year, resistance to this mixture and many other insecticides commonly used against whiteflies was documented (Dennehy et al. 1996, Dennehy et al. 1997, Dennehy and Williams III, 1997), although previous evidence of resistance had been obtained and warnings issued in 1994 (Dennehy et al. 1995). The management crisis of 1995 led to concerted action by the larger agricultural community that culminated in the emergency registration of the IGRs, Knack® and Applaud®. The skilled and conservative deployment of both IGRs in combination with their selective and highly effective chemistries have contributed to a progressive decrease in whitefly infestations and number of insecticide treatments required for their control (Ellsworth et al. 1998, Agnew and Baker 2000, Agnew et al. 2000, Ellsworth and Jones 2000, 2001).

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While the management crisis in central Arizona was being played out, other regions in the southwest were still experiencing good efficacy with Danitol+Orthene and other pyrethroid/organophosphate combinations. An extensive resistance monitoring database for the Imperial Valley provided indications that net gains in susceptibility to insecticides had occurred in 1995 relative to 1993 and 1994 and that Danitol+Orthene remained one of the most potently toxic treatments against *B. tabaci* despite its intensive use (Castle et al. 1996). In light of these apparent differences in susceptibility to insecticides among regional whitefly populations, it seemed worthwhile to quantify putative differences, then examine characteristics of the various regions that might help to explain why and where resistance problems occurred. In pursuit of that goal, adult whiteflies were collected from Imperial Valley, Yuma Agricultural Center and Maricopa Agricultural Center for comparative insecticide bioassays beginning in 1995. The following year, whiteflies from Mexicali Valley were also included in the comparison of regional whitefly populations.

In the present report, responses of MAC-collected *B. tabaci* to Danitol+Orthene will be emphasized over data from other regions, but selected comparisons will be made as points of contrast. Much of the data to be presented was generated from a series of commercial scale evaluations conducted over consecutive years by Ellsworth and colleagues (Ellsworth et al 1997, 1998, Ellsworth and Naranjo 1999). The advantage offered by these evaluations in terms of monitoring resistance to Danitol+Orthene was that the impact of different treatment regimens on whitefly resistance could be readily compared.

Materials and Methods

Susceptibility of adult whiteflies to Danitol+Orthene and other contact insecticide treatments was evaluated by the yellow-sticky card technique. This bioassay technique takes advantage of the natural attraction of adult whiteflies to the color yellow by using sticky-coated yellow cards treated with various concentrations of an insecticide or mixture of insecticides. The 3x5 inch yellow cards are attached to an 8" long plastic stake used as a handle, then held or waved in a crop canopy while rustling the foliage to induce flight. Whiteflies that fly to the yellow card and alight become stuck in the coating and exposed to the particular treatment and concentration previously applied to the card. By holding a pair of cards back-to-back, 2 pairs of cards could easily be held in 1 hand while using the other hand to grasp and shake crop foliage, thus exposing 4 treated cards at each pass. When $\geq$ 50 whiteflies were stuck to each of the cards, they were inserted stake-down into a styrofoam block within an ice chest for transport back to the laboratory. Upon return, each styrofoam block was removed from the transport ice chest and placed in a larger volume ice chest containing 1 gallon of cool water. Covers were placed on each of the incubation chests to allow a 24 h exposure period in a dark and humid environment at room temperature. The next day, mortality of adult whiteflies on each card was determined by examination under a microscope. Any responsive movement to stimuli by individual whiteflies, albeit often sluggish, was scored as alive.

Monitoring of whitefly susceptibility to insecticides was a year-round operation in the Imperial Valley from 1992 through 1995. However, bioassays using Danitol+Orthene did not commence until July, 1994, soon after Section 18 registration was granted in California. As many as 8-10 cotton fields were visited periodically to collect whiteflies on treated yellow cards. Collections were made without knowledge of treatment regimens for specific fields, although all cotton fields in the Imperial Valley were heavily treated with Danitol+Orthene and other pyrethroid mixtures during 1994 and 1995. For example, a random sample of 11 cotton fields in 1994 revealed that an average of 6.6 treatments were applied against whiteflies between June 1 and August 20, and that 3.7 of these were Danitol+Orthene.

The first comparisons of whitefly susceptibility to Danitol+Orthene between Arizona and California were made in October, 1995. Late season collections were made at MAC on 2 dates following reports of poor efficacy with Danitol+Orthene. The following year, 4 collections were made at or around MAC during July, August and twice in September. Beginning with the 1997 season, whiteflies at MAC were collected on yellow-sticky cards from specific treatment plots within a large-scale trial comparing different whitefly control regimens (Ellsworth et al. 1997, 1998, Ellsworth and Naranjo 1999). Two of the regimens involved the use of IGRs as the initial treatment upon attaining the action threshold, i.e., 1 large nymph/disk plus 3-5 adults/leaf. Either Knack or Applaud was used as the first IGR in their respective regimens, to be followed by the 2nd IGR if action thresholds were subsequently attained. In 1997, both the Applaud-first and Knack-first plots required a 2nd IGR treatment followed by a Thiodan®+Ovasyn® treatment and a Vydate®+Curacron® treatment (Naranjo et al. 1998). In both 1998 and 1999, only the first IGR treatment was necessary in the respective Applaud- or Knack-first plots (Ellsworth and Naranjo 1999). A third whitefly control regimen employed conventional chemistry rotated according to recommendations developed for the

1995 season and referred to as '95-IRM' (Ellsworth et al. 1996, 1998). In 1997, 2 treatments of Danitol+Orthene were applied on 20 August and 12 September as well as 4 other conventional treatments applied through the season (Naranjo et al. 1998). Ellsworth and Jones (2001) summarized treatment patterns and whitefly population dynamics in these large-scale studies (1995-2000). Yellow-card bioassays were conducted in the 95-IRM plots, IGR plots and untreated control plots 4 days after the second Danitol+Orthene treatment. Late season pressure was so low in 1998 that not one Danitol+Orthene treatment was applied. In 1999, a single application of Danitol+Orthene was applied September 10 to the 95-IRM plots, with bioassays conducted in the 95-IRM, IGR and untreated control plots 3 days later.

Results and Discussion

Regional differences in whitefly susceptibility to insecticides have been highlighted across Arizona over the past 6-7 years through the efforts of Tim Dennehy and colleagues at the University of Arizona (Dennehy et al. 1995, 1996, 1997, 1998, Dennehy and Williams III, 1997). In the present examination, we observed consistently greater susceptibility to Danitol+Orthene in Imperial Valley whiteflies compared to those collected at and around MAC over a 5 year period. In 1995, differences in susceptibility of whiteflies from the 2 regions was most apparent by the steep and progressive increase in mortality observed in the Imperial Valley whiteflies compared to the flatter response of MAC whiteflies to increasing concentrations of Danitol+Orthene (Fig. 1). At the top concentration of 150 µg/ml of Danitol used in 1995, the mean mortality of whiteflies collected at MAC was just higher than 50%, whereas not a single whitefly survived this concentration out of 17 bioassays conducted in Imperial Valley cotton. The variability in mortality of whiteflies to Danitol+Orthene was greatest at a concentration of 9.4 µg/ml of Danitol for Imperial Valley whiteflies compared to the 2 highest concentrations of 75 and 150 µg/ml of Danitol for MAC whiteflies (Fig. 1). This was potentially an ominous sign in that further applications of Danitol+Orthene at and around MAC could have shifted the phenotypic expression of resistance beyond the 150 µg/ml concentration.

The contrast in susceptibility to Danitol+Orthene between whiteflies from both regions was again observed in 1996, but was more pronounced at the lowest concentrations. There was virtually no increase in mortality of MAC whiteflies through the first 3 concentrations of Danitol+Orthene, whereas mean mortality of Imperial Valley whiteflies at 18.7 µg/ml was 63% compared to 10% at MAC (Fig. 2). However, the mortality responses of Imperial Valley whiteflies in 1996 were flatter at the lowest concentrations compared to 1995. Whereas the mean mortality at 9.4 µg/ml of Danitol was 50±5% in 1995, it was only 32±5% in 1996. In addition, the concentration having the highest variability in mortality increased to 18.7 µg/ml in 1996 from 9.4 µg/ml in 1995 (Fig. 2). At MAC, the highest variability in mortality shifted back to 37.5 µg/ml in 1996. However, the presence of highly resistant whiteflies to Danitol+Orthene was still apparent in 1996 with the relatively high proportions of whiteflies that survived the 3 highest concentrations of 300, 600, and 1200 µg/ml of Danitol+Orthene (Fig. 2).

Although the 1995 and 1996 comparisons between Imperial Valley and MAC revealed much greater susceptibility to Danitol+Orthene in Imperial Valley whiteflies, some erosion occurred between 1995 and 1997. Adult whiteflies in the Imperial Valley proved to be most susceptible to Danitol+Orthene during its second year of use in 1995 (Fig. 3). One possible explanation for this apparent anomaly is that the 1995 cotton season followed the first full year of commercial use of Admire® in Fall, 1994 vegetables and Spring, 1995 cantaloupes. The potential impact of a novel compound such as Admire on pyrethroid/organophosphate-resistant whiteflies could have been to operationally reduce the number of whiteflies normally exposed to conventional insecticides prior to the advent of Admire. With fewer conventional insecticide treatments during Fall, 1994 and Spring, 1995 to select for resistant whiteflies, and with Admire working effectively against both pyrethroid/OP-resistant and susceptible whiteflies, a net gain in susceptibility may very well have been the outcome observed in 1995. However, a significant shift towards reduced susceptibility to Danitol+Orthene was revealed in 1997 and again in 1999 (Fig. 3). In contrast, susceptibility of MAC whiteflies to Danitol+Orthene appeared to be remarkably consistent between 1996 and 1999. Composite dose-mortality bar graphs for each year reveal little deviation from one year to the next (Fig. 4). Notice, however, that relatively large percentages (5-20%) of whiteflies still survived the 600 and 2400 µg/ml concentrations of Danitol+Orthene in bioassays.

Perhaps the best evidence of the continued presence of Danitol+Orthene-resistant whiteflies at MAC comes from rapid changes in the proportion of individuals capable of surviving higher concentrations of Danitol+Orthene following application of this mixture in a cotton field. Our bioassays in 1997 and 1998 detected immediate shifts in susceptibility to Danitol+Orthene following treatment of the 95-IRM plots with this mixture. When viewed in the context of 3 consecutive years of bioassays of whiteflies collected from experimental plots treated either with IGRs, conventional chemistry or left untreated, the impact of the Danitol+Orthene treatments on resident whiteflies was quickly revealed (Fig. 5). The shift to higher proportions of whiteflies surviving bioassays was most evident at the 3 highest concentrations of Danitol+Orthene and occurred 16 September in 1997 and 13 September in 1999 (Fig. 6). An accompanying spike in mortality variability can also be observed on these 2 dates as a wider range of responses to Danitol+Orthene became apparent following the resistance-selecting field treatments in the 95-IRM plots.

Following the management crisis of 1995, whiteflies regained some measure of susceptibility to Danitol+Orthene with the burden of whitefly control shifting to the IGRs beginning in 1996 in Arizona. Bioassay responses of whiteflies from 1996 through 1999 to Danitol+Orthene remained very consistent with the exception of the deviations that occurred soon after treatment with Danitol+Orthene. The rapid increase in frequency of individuals surviving bioassays following treatment makes clear that a significant reservoir of Danitol+Orthene-resistant whiteflies remain in the vicinity of MAC.

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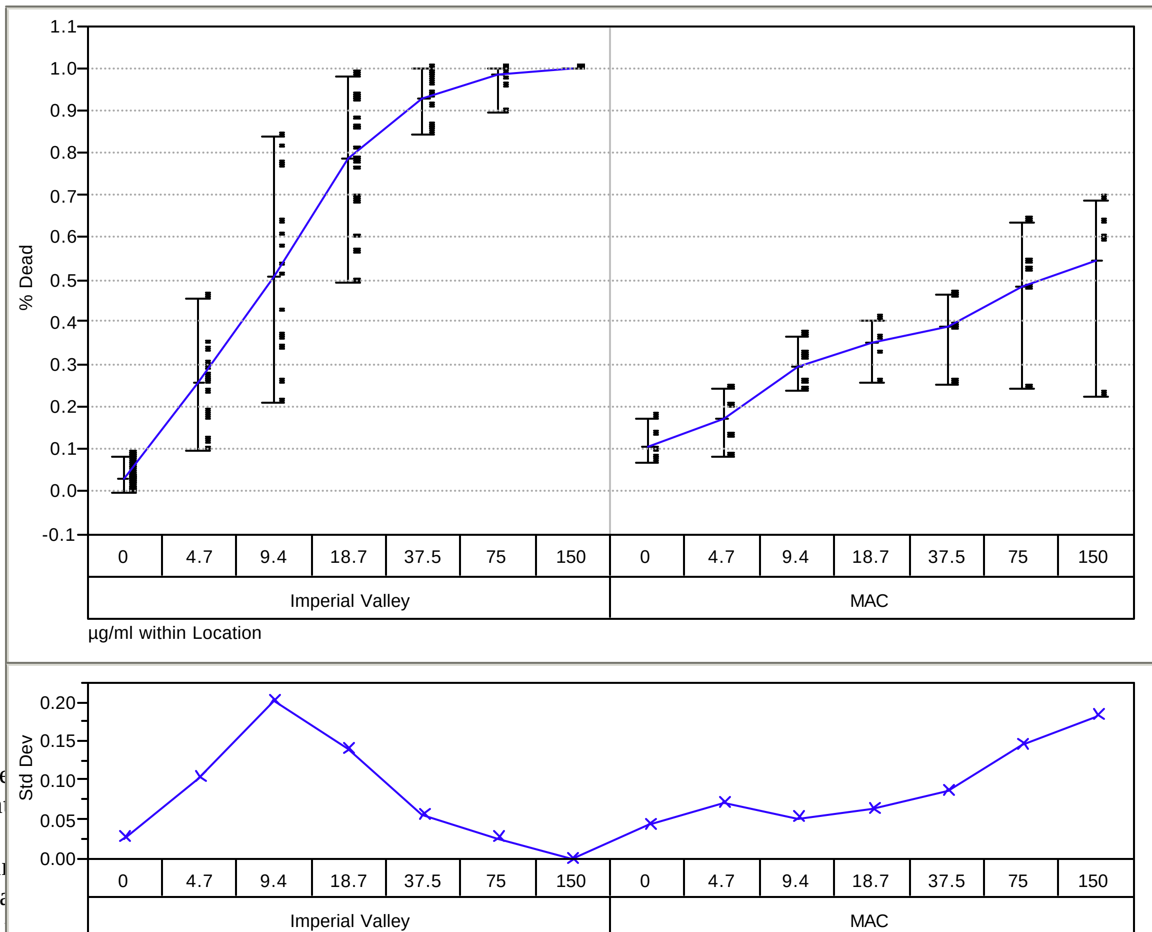

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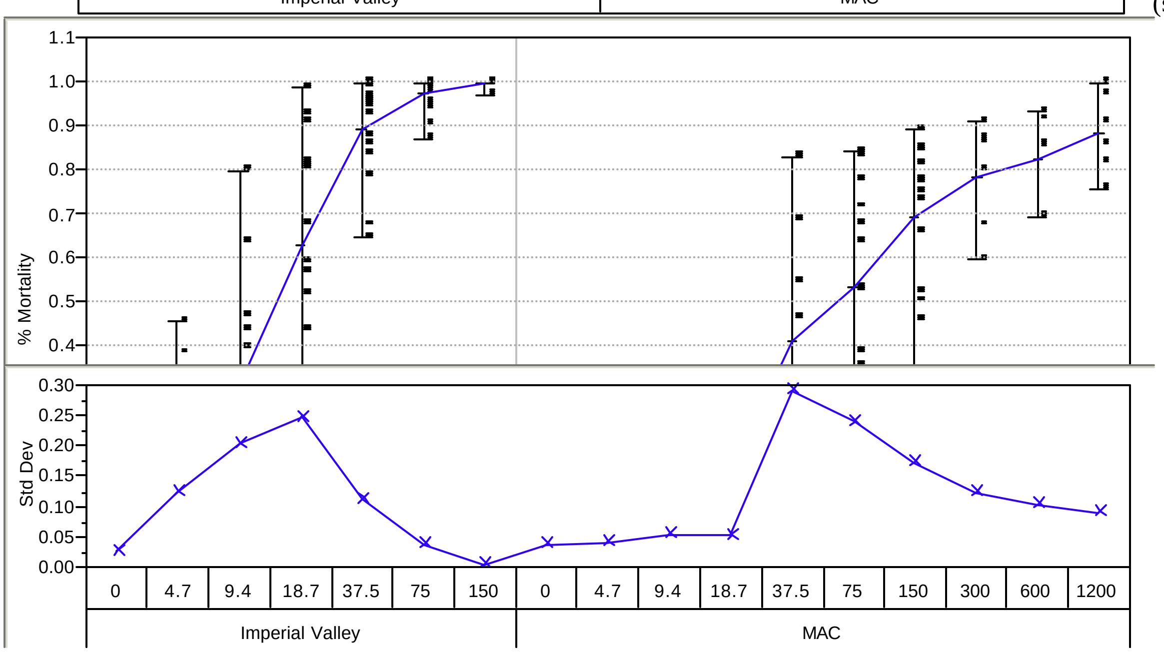


Fig. 2. Relative susceptibility of whiteflies collected on mid to late season cotton in the Imperial Valley, CA and at MAC in central Arizona to Danitol (µg/ml) + Orthene in 1996. Note the increasing variability in mortality at 18.7 µg/ml for Imperial Valley whiteflies compared to 1995, as well as the flat response through the lowest 3 concentrations for MAC whiteflies.

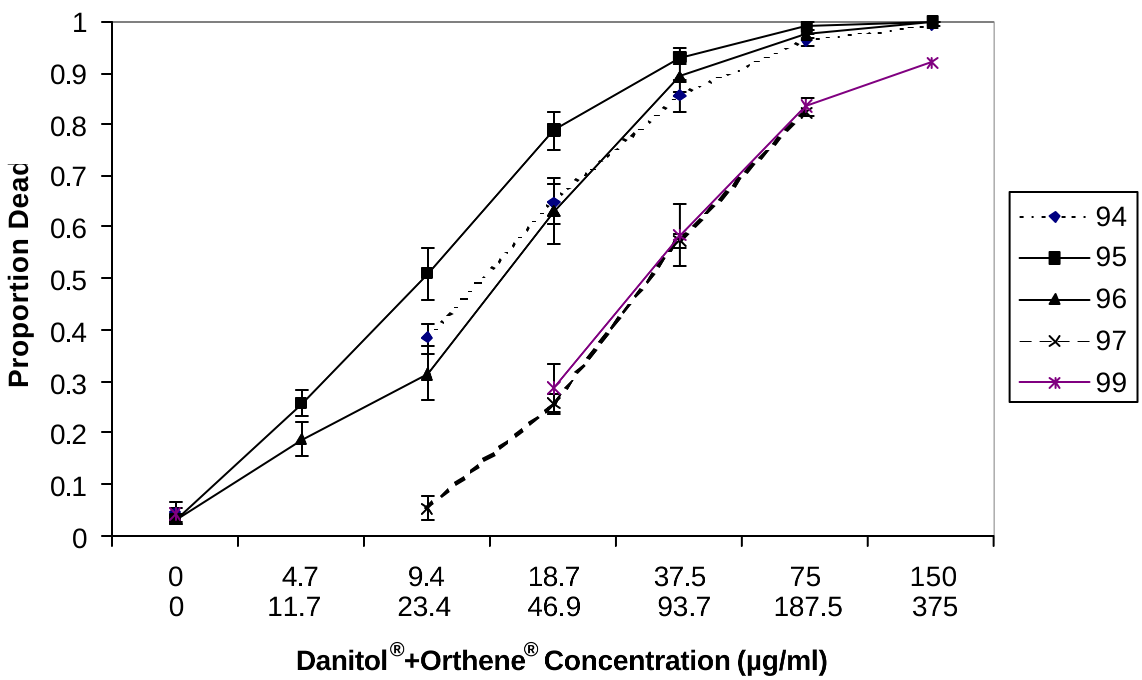


Fig. 3. Composite dose-mortality lines for 5 years of Danitol+Orthene bioassay results in the Imperial Valley. The year of highest susceptibility was 1995, but thereafter response lines shifted to the right indicating the need for higher concentrations of Danitol+Orthene to kill an equivalent number of whiteflies

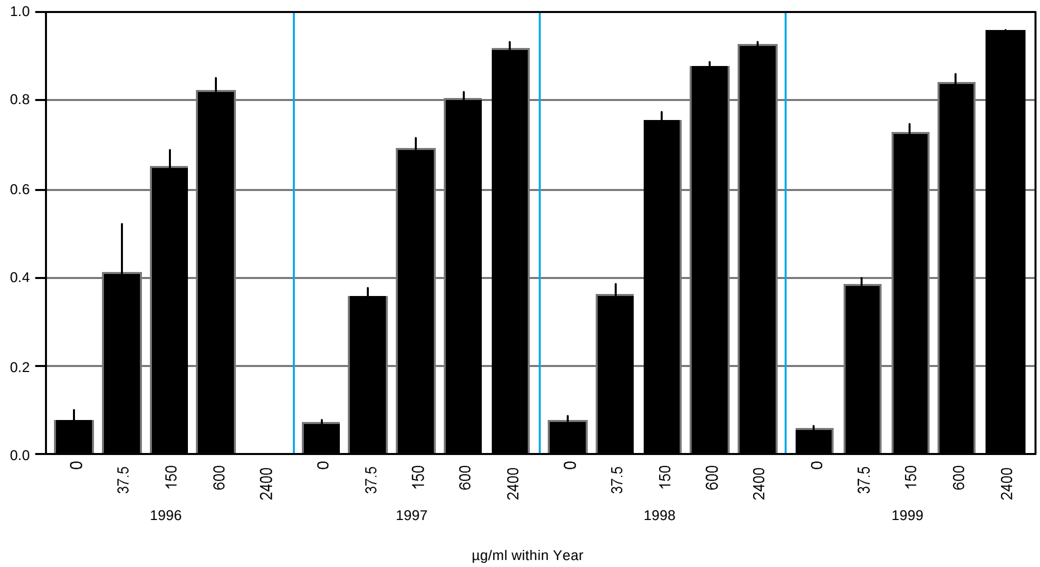


Fig. 4. Composite bar graphs for 4 years of monitoring susceptibility to Danitol+Orthene at MAC showing little variation in mortality (within concentrations) between years. Each bar represents the mean response (+SEM) for an entire season's monitoring data at each concentration. The 2400 µg/ml concentration was not used in 1996. (Y-axis is Proportion Dead).

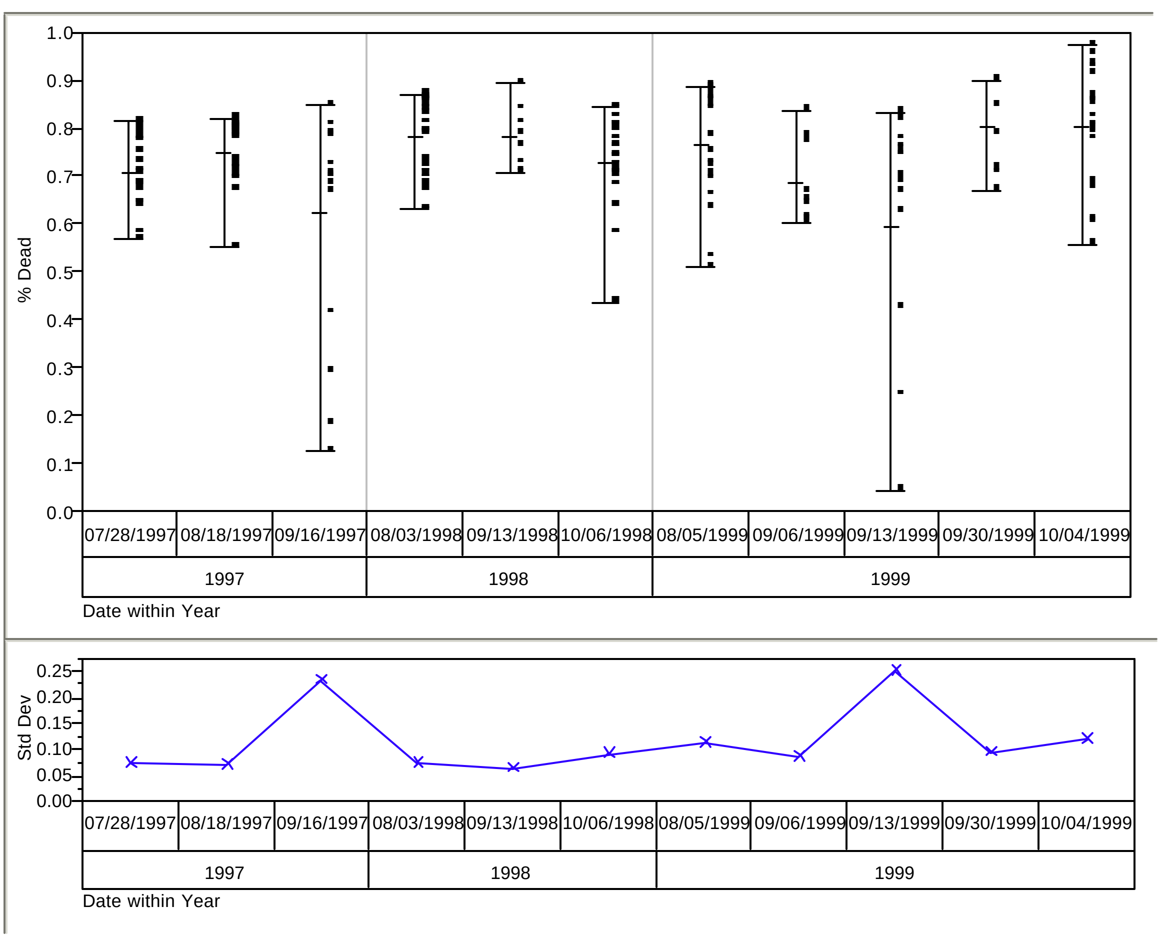
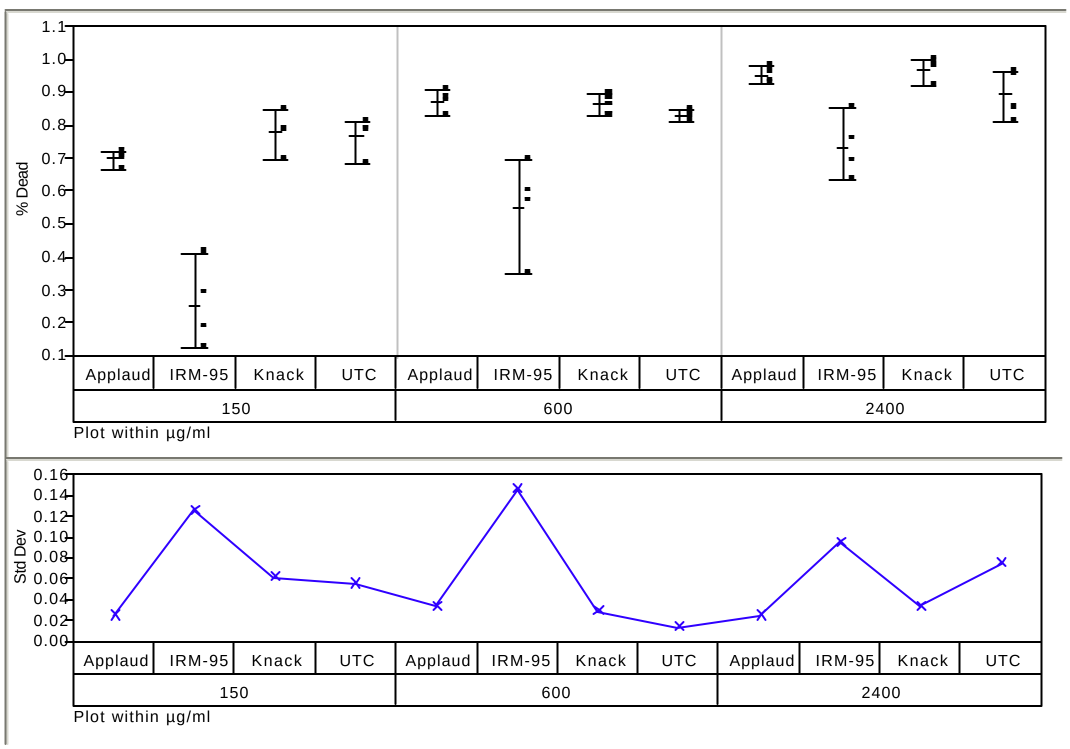


Fig. 5. Mortality distribution at 150 µg/ml of Danitol+Orthene for each of 3 seasons at MAC for all treatment plots combined. The range of mortality is fairly uniform across years and most dates. The exceptions, however, occurred on September 16, 1997 and September 13, 1999 when the ranges of mortality increased as well as the variability as indicated by the standard deviation measure (please refer to Fig. 6). The bioassays conducted on each of these days followed by just a few days Danitol+Orthene treatments applied to the 95-IRM plots. Very similar patterns to the above were also observed at the 600 and 2400 µg/ml concentrations of Danitol+Orthene.

6 (a) September 16, 1997



6 (b) September 13, 1999

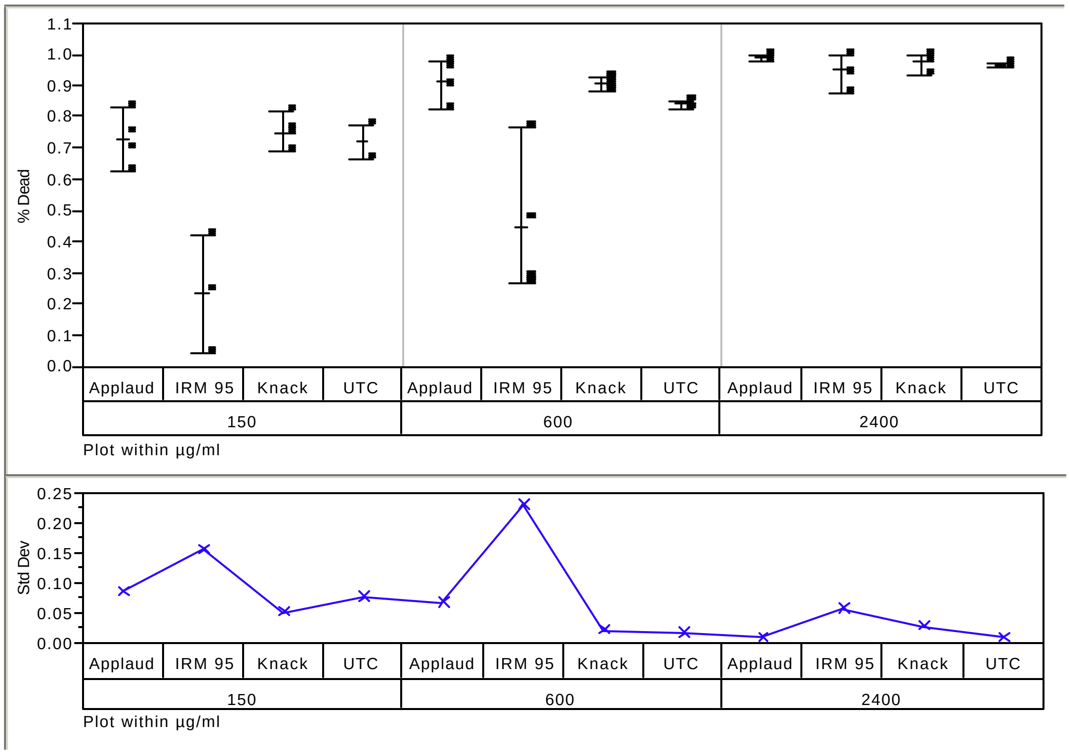


Fig. 6. Bioassay results on 2 dates at 3 concentrations following treatment of 95-IRM plots with Danitol+Orthene. Both figures show reduced mortality of whiteflies sampled from the 95-IRM plots compared to ones sampled from the Applaud, Knack and untreated control plots (UTC).