

Monitoring *Bemisia* Susceptibility to Applaud (buprofezin) During the 1998 Cotton Season

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Abstract

Starting in 1993, we developed a field-based protocol for bioassaying sweetpotato whiteflies (SWF) for susceptibility to buprofezin (Applaud®). Since then, we have monitored Arizona SWF populations (up to 5 regions) for susceptibility to Applaud in four out of the last six seasons. We observed no appreciable decrease in susceptibility. Instead, we have observed an increase in susceptibility of present day whiteflies when compared to populations bioassayed in 1993 and 1996, before any Applaud use in the U.S.. This result, however, is likely related to various procedural changes in the bioassay methodology. Nevertheless, our current estimates of whitefly susceptibility are similar to those obtained from various unexposed populations from around the world and to populations we bioassayed in 1997. Differences between our LC_{50} estimates and those of some other researchers can probably be explained by various procedural differences: 1) method of Applaud application, 2) whitefly stage collected and sources of leaf foliage, and 3) bioassay environmental conditions. Our results also showed each year that Applaud susceptibility does not decline after Applaud application(s) based on commercial paired field comparisons and replicated small and large plot evaluations. In fact, susceptibilities actually marginally increased after an Applaud application. This fact does not alter the recommendation for Arizona to limit Applaud use to one time per crop season, but does provide hope for the development of a sustainable use pattern even if usage continues on non-cotton hosts (i.e., on melons and vegetables under Section 18). Given the tremendous value of this mode of action, however, commodity groups should work together wherever possible to coordinate the usage of this and other valuable compounds so that whitefly generations are not successively exposed to this product.

Introduction

Since receiving the section 18 emergency exemption for use of Applaud® (buprofezin) against the sweetpotato whitefly (SWF) [*Bemisia argentifolii* (Bellows & Perring), a.k.a. silverleaf whitefly] in 1996, its field efficacy against Arizona SWF populations has not waned (Ellsworth et al. 1997, 1998a, b; Ellsworth & Naranjo, *this volume*, Naranjo & Ellsworth, *this volume*). However, the potential for resistance development cannot be ignored (Horowitz & Ishaaya 1994, Cahill et al. 1996, Simmons et al. 1997). The development of an insecticide resistance management (IRM) program is important when attempting to protect such a valuable mode of action (Horowitz et al. 1994, Ellsworth et al. 1996). Monitoring field populations can be one component of these programs (Horowitz & Ishaaya 1992, Cahill et al. 1996, Simmons et al. 1997).

Applaud is an insect growth regulator, active primarily against the nymphal stages of *Bemisia*. More specifically, it inhibits chitin synthesis, interrupting the normal physiological process of molting (Uchida et al. 1985, Yasui 1993, Ellsworth & Diehl 1996). Thus, susceptibility monitoring is best examined in the immature stages (i.e., nymphs). Monitoring actual field populations for susceptibility to Applaud can be difficult, requiring the maintenance of nymphal stages on host plant material. Various methods have been used to determine susceptibility of field-collected SWFs to Applaud: treatment of field-collected adults and observation of progeny formation and development (Horowitz and Ishaaya 1992); treatment of nymphal progeny produced by field-collected adults (Simmons et al. 1997, Toscano et al. 1998); and treatment of nymphs hatched from field-collected egg-infested leaves (Yasui et al. 1997a, b). Techniques which depend on adult collections and progeny exposures to Applaud require extensive culturing of insect and host material in the greenhouse and/or laboratory. In the current study, we used the methodology developed by Yasui et al. (1997a, b), which depends on assaying *in situ* whiteflies on terminal field foliage.

In 1996, we began monitoring field SWF populations for susceptibility to Applaud from multiple state locations (Yasui et al. 1997a, b). We saw no appreciable decrease in susceptibility when compared against a baseline estimate determined in 1993 (3 years prior to any Applaud use in the U.S.). Nonetheless, a proactive, industry-supported component of Arizona's IRM is to restrict the number of Applaud applications to once per season (Ellsworth et al. 1996). Since its introduction in 1996, over 160,000 A of cotton have been treated with Applaud (Ellsworth 1999). Starting in 1998, Applaud use was permissible in melon crops under a section 18 (Palumbo et al. 1999).

The objective of the current study was to determine Applaud susceptibility of SWF populations from multiple cotton-growing regions in Arizona in 1998. By comparing our 1998 results with baseline estimates determined for Arizona SWF populations (Yasui et al. 1997a), we can determine whether there has been any appreciable decrease in susceptibility to Applaud. In addition, we provide an explicit description of our methods for evaluating Applaud susceptibility of any field population of SWF, without the extensive SWF culturing requirements of other techniques.

Materials and Methods

Field collection

From randomly selected plants, we collected the 2nd mainstem leaf below the terminal. There were four criteria for leaf selection: 1) the first terminal leaf should be quarter-sized (a way in which to approximate the age of the 2nd terminal leaf), 2) the leaf should be young enough to harbor only eggs (preferably < 2 days old or white in appearance), 3) the leaf should not have an under or over abundance of eggs (preferably 50–100 eggs / leaf), and 4) the leaf should be large enough for adequate trimming (to 3 x 4 cm). However, when the mainstem terminals were not an adequate source for SWF eggs (e.g., at crop cut-out), we harvested leaves from other locations of the plant (e.g., actively-growing laterals) as long as they conformed to the criteria above.

When harvesting the leaf of interest, we placed the scissors at the base of the petiole so as to yield the longest petiole possible. Harvested leaves were placed in plot (field) specified Ziploc[®] bags and stored within an ice cooler. The Ziploc bags were placed atop a thin sheet of Styrofoam in order to avoid direct contact with the ice and transported back to the laboratory for preparation.

Construction of leaf bioassays

Collected leaves were trimmed to 3 x 4 cm (width x height) for bioassay arena uniformity using a paper template centered against the main vein of the leaf. Immediately after trimming, the leaves were placed into 20 ml scintillation vials (Kimble[®] glass, Inc., Vineland, N.J.) and filled with tap water. This specific make of vial has the required orifice diameter for supporting the leaf petiole and minimizing evaporation. For leaves with shorter petioles, a wick made of absorbent cotton was loosely wrapped around the petiole of the leaf to permit water absorption and provide support. This was usually done immediately upon return from the field; however, uncut leaves could be held in Ziploc bags at 15°C overnight.

Preparation of leaf bioassays

All vials were maintained in an incubator at $25 \pm 1^\circ\text{C}$, and $80 \pm 10\%$ R.H. If possible, we maintained all individuals

from a specific site (field) within the same incubator.

While not a requirement of the procedure, within 3 days after the date of collection, the number of eggs on each leaf was counted. This facilitated the selection of leaves for the bioassay (see next section). Leaves with 50–100 eggs/leaf were preferentially chosen for use in the bioassay, because the resulting number of nymphs from such leaves were easier to observe following treatment. At the same time, any predators or whitefly nymphs were removed using an insect pin, taking special care not to score the surface of the leaf. This provided for as uniformly-aged cohort of SWF nymphs as possible. We did not count hatched or overly mature eggs (very brown), as they would hatch too soon, before the start of the bioassay. On the morning of the assay, each leaf was re-examined and large nymphs (> 1st instar) and any predators were removed.

We divided the leaves so as to establish approximately the same number of SWF subjects within each treatment dose (ca. 600–700 eggs). This usually resulted in a final assay count of about 500 SWF subjects/dose. Where possible, leaves with egg densities less than 50 or more than 110 were rejected. In most cases, the initial whitefly egg densities averaged about 75 / leaf replicate. At least 4–5 leaf replicates/dose were used. At least 2 additional leaves/site were reserved as replacements to leaves lost prior to the start of the assay. On the day of treatment, leaves were culled-out if they were necrotic, torn, perforated, or otherwise physiologically-compromised.

Preparation of Applaud and bioassay treatment

A 0.01% Triton[®] X-100 (Sigma, St. Louis, MO.) wetter was prepared by mixing 0.2 ml Triton X-100 within 2000 ml of water. Serial dilutions of Applaud were prepared from an initial stock solution of 200 ppm. The Applaud stock solution was made with 0.1 grams of Applaud 70 WP in 350 ml of the 0.01% Triton X-100 wetter. From the 200 ppm stock solution, 7 serial dilutions were made: 100, 30, 10, 3, 1, 0.3 and 0.1 ppm (control = 0.01% Triton X-100). Applaud preparations were used immediately (i.e., preparations were not stored).

Insecticide application was made 7 days (@ 25 °C) after the date of field collection. This period resulted in mostly settled 1st instar nymphs, the target of our bioassay. A household hand-pump sprayer was used to apply the Applaud preparations. The sprayer uptake was placed within a 500 ml Fleaker[™] (Baxter Diagnostics Inc., McGraw Park, IL.) containing the insecticidal solution, and applications were made within a well-ventilated hood. The absorbent cotton wicks were removed from the petioles, if present, prior to insecticide application. A custom petiole holder positioned leaves for spraying both sides of the leaf. Each side was sprayed 10–12 times or until run-off from a distance of about 1 ft. All leaf replicates per concentration were sprayed at a time, starting from the lowest and finishing with the highest Applaud concentration. Immediately following application, leaves were propped-up on filter papers to allow drying (takes ca. 10–30 minutes under ambient room conditions). Once the treated leaves were dry, absorbent cotton was wrapped around each petiole, and then placed back into their original vials.

Maintenance and conditions of the bioassay

The treated leaves were maintained in a large rearing room at 25 ± 1°C and 40 ± 10 % R.H, separate from the untreated leaves maintained in incubators. We did this to avoid the possibility of contamination via the described vapor phase of buprofezin (DeCock et al. 1990). Leaves were periodically checked, re-watered, and predators and adult whiteflies were removed if present.

Observation of treated leaves

Observations were made 10 days following Applaud treatment using a dissecting microscope. Leaves were scored for SWF mortality (i.e., the number dead and number alive by instar). Most nymphs dying of Applaud were amber in color, sometimes showing obvious inability to molt. Individuals appeared to have a “double-skin” or only partially cast-off old cuticle. Mycetomes were often off-colored (e.g., amber). Larger instars (3rd & 4th) were often scored as alive (i.e., these nymphs successfully molted), if they did not display the above symptoms. Mortality by Applaud was sometimes very subtle, even in the younger instars (1st & 2nd). They were characterized as having a “cloudy” cuticle, sometimes with more apparent (i.e., darkened) pin-point eye spots. In contrast, the younger instars scored as alive had transparent cuticle (i.e., not “cloudy”). Other apparent causes of mortality were observed (e.g., predation and parasitism), and these individuals, or entire leaf replicates, were discarded from all analyses.

A bioassay such as this one with subtle mortality, small subjects, and large numbers requires significant training of the samplers. We wanted to compare “scores” of various samplers to test the effectiveness of our sampler training and any potential sampler bias or error. We grouped samplers as either 1st scorers or 2nd scorers. The 1st scorer read the assay, and then the 2nd scorers re-read the very same assay, usually on the same day. Otherwise, completed bioassays were held in cold storage until re-reading by the 2nd scorers. The 1st scorers had more prior experience reading assays. Due to logistical constraints, in many cases it was not possible for the second reader to re-read all leaf replicates.

Field Populations

Samples were collected from 5 cotton growing regions: Stanfield, Yuma, Buckeye, Coolidge, and Maricopa (Maricopa Agricultural Center, two locations) (table 1). From each region, we collected leaves from a field that had a prior Applaud application in 1998 (designated as days after treatment, DAT), and from another field that had not received an Applaud application in 1998 (designated UTC). We selected fields that had not been sprayed with Applaud more recently than 21 days (>30 days preferable) or with conventional insecticides more recently than 10 days (>20 days preferable). Following these safeguards and because we were always collecting leaves from the actively-growing terminal, our leaves were likely to be free of any significant insecticide residues. At all sites, except Stanfield, collections were made on the same day. The Stanfield UTC was collected prior to the Applaud application, and then we returned to this same field 21 DAT and collected our post-treatment sample. The Stanfield site was collected from 4, 5-acre replicated experimental plots within a 41 A field. At the first Maricopa (MAC) location, leaves were collected from each of 4 field replicates from a small plot (0.033 A) experiment. Three populations were selected: one that was untreated (UTC), one from plots treated once with Applaud (33 DAT), and one from plots treated twice with Applaud + endosulfan (33 DAT2). The second MAC site was a large plot (0.33 A), replicated experiment located on the demonstration farm with both untreated (UTC) and treated (42 DAT) plots. The other post-treatment samples were from: Yuma, 34 DAT; Buckeye, 42 DAT; and Coolidge, 39 DAT.

Historical Comparisons

To determine whether there has been any decrease in susceptibility to Applaud in Arizona SWF populations, we compared 1998 results with past seasonal observations made by our laboratory (Yasui et al. 1997a). Our bioassay methodology has differed slightly among seasons. In 1993, we used the formulation Applaud 40 SC in a 0.125% Kinetic[®] wetter (Helena Chemical, Memphis, TN) and maintained the bioassay at higher humidities (70–80% R.H.). In 1996, we began using Applaud 70 WP in 0.01% Triton X-100 wetter (as in subsequent years) and maintained our bioassays at higher humidities (60–70% R.H.) than in the years that followed (i.e., 1997 and 1998 used 40% R.H.).

Data analysis

Bioassay data were analyzed using the probit model by POLO-PC program (LeOra Software 1987). Population responses were considered significantly different if their 95% fiducial limits did not overlap.

Results

1998 regional LC₅₀ estimates

All 1998 LC₅₀'s are reported in ppm with 95% fiducial limits and are plotted in figure 1.

The Stanfield site before and after treatment with Applaud (21 DAT) produced populations with LC₅₀ estimates of 4.5 ppm (3.2, 5.9) and 2.7 ppm (1.9, 3.5), respectively. The susceptibility of the 21 DAT population was greater than the UTC population, but not significantly (P>0.05) (fig. 1). Compared to Stanfield, Yuma had significantly lower LC₅₀ estimates of 1.0 ppm (0.7, 1.4) and 0.9 ppm (0.3, 1.9) for the UTC and 34 DAT populations, respectively (P<0.05), regardless of treatment with Applaud. Buckeye had similar LC₅₀ estimates to Yuma of 2.2 ppm (1.6, 2.8) and 1.3 ppm (0.6, 2.0) for the UTC and 42 DAT populations, respectively, regardless of Applaud application. Coolidge populations also had very similar LC₅₀ estimates of 1.1 ppm (0.7, 1.4) and 0.8 ppm (0.5, 1.1) for its UTC and 39 DAT, respectively. The UTC and 42 DAT populations from the MAC large plot site also had very similar LC₅₀ estimates of 2.2 ppm (1.5, 3.1) and 1.9 ppm (1.3, 2.6) for its UTC and 42 DAT populations, respectively. Results from the MAC small plot site mimicked those of the large plot site with no significant differences among LC₅₀ estimates of the UTC, 33 DAT and 33 DAT2 populations: 1.2 ppm (1.0, 1.4), 1.5 ppm (1.2, 1.7), 1.3 ppm (1.0, 1.7), respectively. The additional Applaud

spray in the 33 DAT2 plots did not significantly change the LC_{50} parameters of the SWF population.

Overall, the consistency of the response in 1998 was striking (fig. 1). Most LC_{50} estimates fell between 1 and 3 ppm. One population, however, stood out as slightly higher (4.5 ppm). The Stanfield pre-treatment population was significantly less susceptible than all other regional populations tested ($P < 0.05$) (fig. 1). Furthermore, the 21 DAT population was significantly less susceptible than some of the populations in Yuma, Coolidge and MAC. The Stanfield pre-treatment population was the first one collected in 1998 (9/14), and it was from a very late maturing cotton field (planted 6/1). In respects to LC_{50} estimates, the lowest LC_{50} estimates were found for post-treated populations from Yuma (34 DAT) and Coolidge (39 DAT). The Yuma UTC population was statistically more susceptible than the Buckeye and MAC large plot UTC populations ($P < 0.05$). Coolidge 39 DAT was significantly more susceptible than MAC large plot 42 DAT and MAC small plot 33 DAT ($P < 0.05$). However, the magnitude of these differences may be biologically and ecologically trivial.

1998 regional probit slope estimates

Slopes of the probit regressions (\pm S.E.) appear in figure 1. Probit regression lines are plotted in figures 2–7. All slopes fell between 1.4 and 2.8, with most falling within the range of 1.4–2.2: Stanfield, 2.2 (± 0.07) and 2.08 (± 0.15) for UTC and 21 DAT populations, respectively; Yuma, 1.52 (± 0.06) and 1.72 (± 0.09) for UTC and 34 DAT populations, respectively; Buckeye, 1.50 (± 0.09) and 1.53 (± 0.06) for UTC and 42 DAT populations, respectively; Coolidge, 1.62 (± 0.07) and 1.77 (± 0.04) for UTC and 39 DAT populations, respectively; MAC large plot, 2.5 (± 0.15) and 2.07 (± 0.1) for UTC and 42 DAT populations, respectively; and MAC small plot, 2.21 (± 0.11), 2.0 (± 0.1) and 2.0 (± 0.09) for UTC, 33 DAT and 33 DAT2 populations, respectively.

Comparatively, the Maricopa large plot UTC population had a considerably steeper slope than all other regional populations tested (fig. 1), possibly as result of the high amount of control mortality (see table 1). Furthermore, the slope of the MAC large plot population (42 DAT) was somewhat larger than the Yuma (34 DAT), Buckeye (42 DAT), or Coolidge (39 DAT) slopes. Also, Yuma (34 DAT), Buckeye (42 DAT), and Coolidge (39 DAT) populations had smaller slopes than both MAC and Stanfield post-treatment populations.

Sampler training and variation

The 2nd scoring LC_{50} estimates were never significantly different from their 1st scoring counterparts ($P < 0.05$) (fig. 1). There was a weak pattern observed between most 1st and 2nd scorings. In 7 out of 12 comparisons, the 2nd scorings produced larger LC_{50} estimates than that of the 1st scorings. The fiducial limits followed no particular pattern, though in one instance (Yuma 34 DAT), the 2nd readings lead to a much narrower fiducial interval. Differences in slopes between the two readers seemed more pronounced, but without a consistent pattern.

Historical comparisons

When comparing our 1998 (fig. 1) and 1997 (fig. 8) Buckeye LC_{50} estimates, there appears to be no pattern of decline in susceptibility to Applaud. On the contrary, both the 1998 UTC and 42 DAT populations were significantly more susceptible than the 1997 UTC Buckeye population ($P < 0.05$). Compared to 1996 SWF populations sampled within the same region as Buckeye (Peoria) (Yasui et al. 1997a) (fig. 10), the 1998 Buckeye SWF populations were more susceptible to Applaud (but not always significantly).

The same relationship between 1997 and 1998 LC_{50} estimates existed for the Coolidge SWF populations (fig. 1 & 8). Furthermore, the 1998 Coolidge SWF populations were significantly more susceptible to Applaud than populations from a similar area (Eloy) (Yasui et al. 1997a) in 1996 ($P < 0.05$) (fig. 10).

Maricopa SWF populations have been monitored for susceptibility to Applaud since 1993, though under slightly different conditions. When comparing our 1998 Maricopa (42 DAT) LC_{50} estimates to Maricopa UTC estimates reported for 1993, 1996 (fig. 10) (Yasui et al. 1997a, b), and 1997 (fig. 8), we observed no decrease in susceptibility (fig. 1 & 9). Furthermore, on most sample dates, the 1998 Maricopa UTC populations appeared significantly more susceptible to Applaud when compared to the 1993 and 1996 populations ($P < 0.05$). This trend was repeated in additional comparison between the LC_{50} estimates of 1993 and 1996 against those of 1998 (fig. 1, 8, 9 & 10).

Discussion

Starting in 1993, three years before any buprofezin use in this country, we began development of a field-based monitoring method for measuring SWF susceptibility to Applaud on cotton. While refining our methods, we established a pre-introduction Applaud susceptibility baseline. In addition to fully describing our bioassay methodology, the main goal of the current project was to determine whether there has been any appreciable change in susceptibility to Applaud since its wide spread use in cotton-growing regions of Arizona. In view of the volume of data collected from five regions of Arizona in 1998, there has been no apparent decrease in SWF susceptibility to Applaud. In many cases, the 1998 LC_{50} estimates were significantly lower than those measured in 1993 and 1996 (Yasui et al. 1997a, b), though similar to those of 1997 (Yasui et al., unpubl. data) (fig. 9). This ostensible recent increase in susceptibility is likely less related to actual changes in the populations and more related to procedural changes between 1993, 1996 and the last two years. In 1993, we bioassayed SWFs using the originally intended formulation of Applaud (40 SC), and we used a different wetting agent (Kinetic). In both 1993 and 1996, we held the bioassays at higher humidities (60–80% RH), whereas in 1997 and 1998 we have standardized the bioassay at 40% RH. Yasui et al. (unpubl. data) has shown that humidity and/or other environmental conditions can significantly influence the probit parameters of identical populations.

Comparatively, our 1998 baseline estimates (fig. 1) fall within the range of most estimates determined for other SWF field populations worldwide. Baseline LC_{50} estimates (95% F.L.) of field SWF populations with no prior exposure to buprofezin (Applaud) for the cotton growing regions of Pakistan and Israel were 0.53 ppm (0.46, 0.62) (Cahill et al. 1996) and 6.4 ppm (2.5, 10.0) (Horowitz et al. 1994), respectively. Prior to wide spread use of Applaud in California, SWF population susceptibility to Applaud was determined for populations in Imperial Valley (2.3–8.0 ppm), Palo Verde Valley (3.0–5.0 ppm) and San Joaquin Valley (2.6–6.5 ppm) (Toscano et al. 1998). Prior to wide spread Applaud use in Arizona, a statewide bulked 1996 LC_{50} value of 0.8 ppm (0.6, 1.0) was reported by Simmons et al. (1997), and Yasui et al. (1997a) established baselines for 1993 and 1996 of 6.8 ppm (3.8, 10.6) and 8.0 ppm (5.6, 10.6), respectively. The difference between the Arizona LC_{50} estimates (Simmons et al. 1997, Yasui et al. 1997a) may be in part due to the method of Applaud application (e.g., as much as a 13-fold difference in LC_{50} 's from dip versus spray application; Yasui et al., unpubl. data). Moreover, the only other SWF population worldwide with a significantly smaller LC_{50} estimate than ours (Pakistan) was treated by the dipping method (Cahill et al. 1996). The other study which applied Applaud as a spray directly on nymphs (California) generated baseline LC_{50} estimates similar to ours (Toscano et al. 1998). Interestingly, the other study reporting an LC_{50} baseline similar to ours (Israel) exposed adult females to leaves that had been sprayed with Applaud and observed progeny formation (Horowitz and Ishaaya 1992), a methodology quite different from all other researchers. In a prior study, Ishaaya et al. (1988) showed that Applaud can affect embryogenesis and progeny formation and development, thus a valid indicator of Applaud activity. However, they further stated that Applaud only affects embryogenesis (presumably only through the adult) and the larval stages of SWF (i.e., no direct mortality to adults or oviposited eggs) (Ishaaya et al. 1988). Thus, even the Israeli procedure depends on observing the nymphal stages of SWF. Another possible source of variation could be treating field-oviposited progeny on *in situ* foliage, as opposed to treating progeny produced by field-collected adults on greenhouse foliage (Yasui et al., unpubl. data). Furthermore, direct testing of field-oviposited progeny may be more indicative of field population response to Applaud and less subject to SWF culturing bias. Our laboratory is continuing to study the influence of various methodologies on observed susceptibility (i.e., mortality).

There are limitations to our current bioassay procedure such as: reliance on good host quality and availability, adequate egg densities, possible influence of other crop chemicals, and problems with predation/parasitism or leaf-feeders (e.g., cotton leafperforator). The main benefit of the other resistance monitoring procedures is the use of “clean” greenhouse foliage and better cohort uniformity (Horowitz and Ishaaya 1992, Simmons et al. 1997, Toscano et al. 1998). The largest constraint on our bioassay procedure was obtaining adequate host plant quality. In addition, our methods depend on adequate levels of actively-ovipositing whiteflies in the field, but has the benefit of eliminating the direct handling of whiteflies. When using greenhouse-grown plants, one can more easily maintain its quality and ensure its “cleanliness” (i.e., from insecticide residues). When field-collecting adults, however, whiteflies must be handled directly through various vacuuming or other aspiration techniques. This could potentially impact adult behavior. Additional handling is required when caging and culturing these same adults for their progeny. Furthermore, adult-based methods ultimately bioassay the F1 progeny of the field population rather than the *in situ* popula-

tion present in the test field as in our bioassay system. Moreover, in the comparison between the first and second readers, we confirmed our bioassay's efficacy and repeatability. Essentially, there were no major differences between the two sets of readers in the 1998 LC_{50} estimates.

The implementation of an IPM-IRM strategy (e.g., restricting use of buprofezin to once per growing season; i.e., one SWF generation) has been essential for maintaining buprofezin's (Applaud) efficacy in Israel (Horowitz and Ishaaya 1992, 1994). However, resistance has reportedly begun to build for other chemistries similarly restricted in Israeli SWF populations (in spite of their IRM program), leading some to suggest that Arizona SWF populations may begin to develop resistance to this chemistry within 5 years of its commercial introduction (Simmons et al. 1997). Furthermore, a 6.8-fold decrease in susceptibility to Applaud in Arizona SWF populations was measured between 1996 and 1997 (Dennehy, unpubl. data). In contrast, our 1998 and earlier results did not detect any reduction in SWF susceptibility to Applaud. In fact, the work reported here would suggest that our current IRM program (Ellsworth et al. 1996) has been, so far, successful in thwarting or forestalling decreases in susceptibility to Applaud as well as to pyrethroids (Castle et al. 1998, Dennehy et al. 1998, Ellsworth et al. 1998a).

An interesting observation in the current and past studies (Yasui et al. 1997a, b) is that increasing the number of Applaud applications against SWF in one season did not decrease susceptibility in small plot studies, as opposed to that reported in Israel populations receiving more than 2 applications in one season (Horowitz & Ishaaya 1994). Given the importance of this unique mode of action against whiteflies and the ostensible success of our current program, however, Arizona still recommends restricting Applaud use to once per crop season. Moreover, considering the fact that Applaud will be used in non-cotton crops (i.e., vegetables and melons) under a section 18 emergency exemption in 1999, the potential of multiple SWF generations being exposed to Applaud increases. As a consequence, a multi-commodity IRM protocol is under development to ensure that successive SWF generations will not be exposed to Applaud (Palumbo et al. 1999). This process will be important to sustaining the long-term efficacy of this valuable IGR in Arizona.

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Table 1. Summary information for 1998 population bioassay locations. Field location is the town of origin of the population. Applaud Treatment Status indicates how many days since field treatment with Applaud, if any (* denotes the summary information for the second scorer of the bioassays). Reps is the number of leaf replicates assayed. Control Mortality is defined as the (total dead * 100) / total subjects for the Triton X-100 control treatment.

Field Location	Applaud Treatment Status	Collection Date	Observation Date	Reps	Total Subjects	Control Mortality (%)
Stanfield	Pretreat	14-Sep	1-Oct	4	8711	23.9
Stanfield	21 DAT	6-Oct	23-Oct	8	2134	36.2
Stanfield	21 DAT*				1633	18.8
Yuma	UTC	21-Sep	8-Oct	8	3352	17.8
Yuma	UTC*				3384	16.7
Yuma	34 DAT	21-Sep	8-Oct	8	1927	16.1
Yuma	34 DAT*				1960	15.7
Buckeye	UTC	18-Sep	6-Oct	10	1628	14.1
Buckeye	UTC*				1620	13.9
Buckeye	42 DAT	18-Sep	6-Oct	5	5001	9.6
Buckeye	42 DAT*				4913	9.6
Coolidge	UTC	21-Sep	8-Oct	5	3050	12.4
Coolidge	UTC*				3227	7.9
Coolidge	39 DAT	21-Sep	8-Oct	4	11257	16.5
Coolidge	39 DAT*				11413	17.5
Maricopa (large)	UTC	17-Sep	5-Oct	8	3390	36.2
Maricopa (large)	UTC*				3433	31.4
Maricopa (large)	42 DAT	17-Sep	5-Oct	8	3035	15
Maricopa (large)	42 DAT*				2955	16.7
Maricopa (small)	UTC	29-Sep	16-Oct	8	3189	11.1
Maricopa (small)	UTC*				3080	11.9
Maricopa (small)	33 DAT	29-Sep	16-Oct	8	2788	9.6
Maricopa (small)	33 DAT*				2963	9
Maricopa (small)	33 DAT2	29-Sep	16-Oct	8	2782	15.5
Maricopa (small)	33 DAT2*				2801	19.1

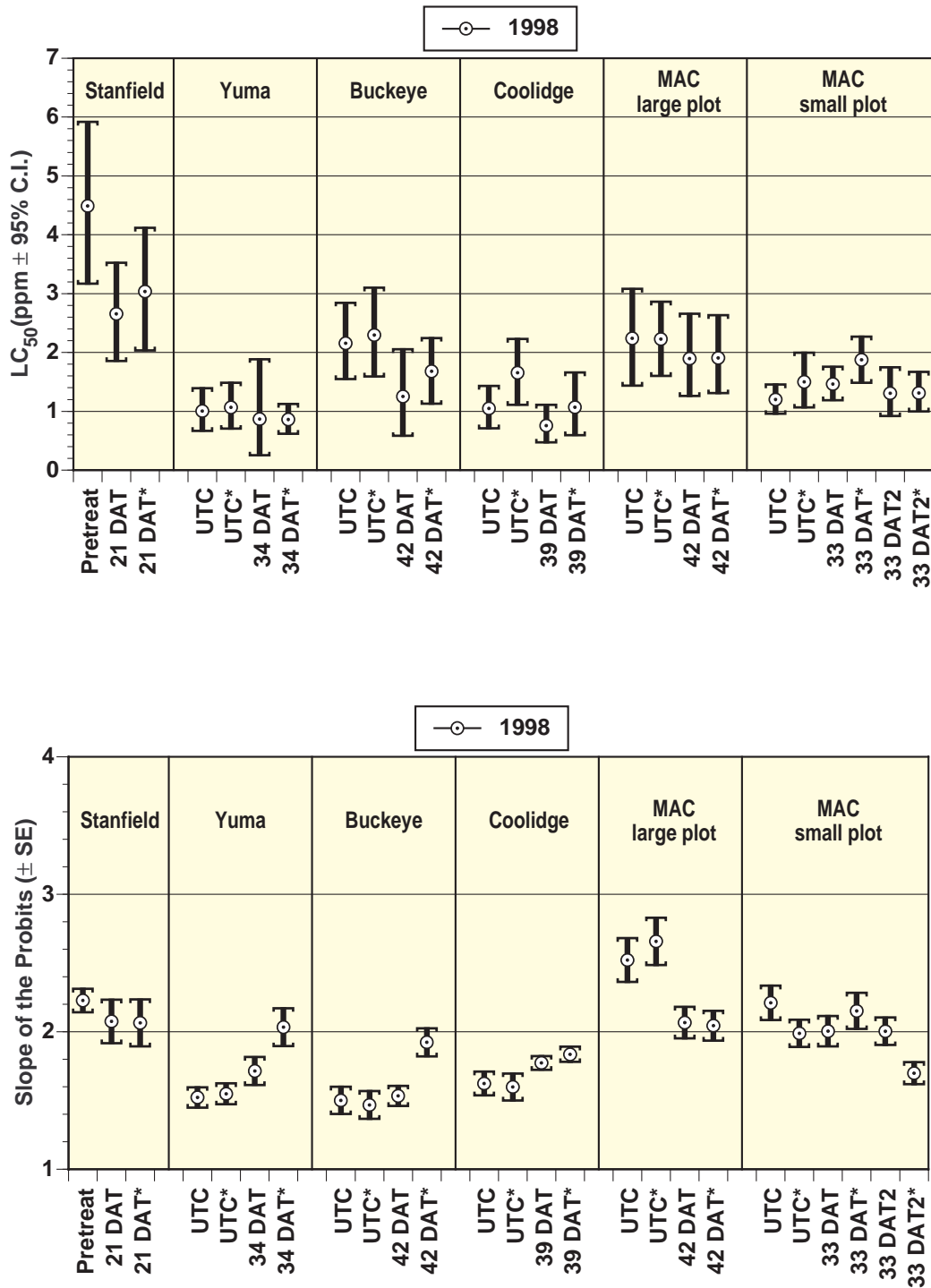


Figure 1. Probit parameters, LC_{50} ($\pm 95\%$ C.I.) (above) and slopes ($\pm SE$) (below), for Arizona populations in 1998. All populations were collected within a 15-day period starting in September. For the Stanfield population, a single site was sampled before ('pretreat') and after field treatment with Applaud (21 DAT). For the remaining sites, field populations were collected from paired fields (or plots in Maricopa), one treated and one not treated ('UTC') with Applaud in 1998. The Maricopa sites were replicated field plots that were either large (0.33 A) or small (0.033 A) in size. At the latter site, an additional population was tested from plots treated twice with Applaud. DAT = days after treatment of the field with Applaud. '*' = second readings of the same bioassays.

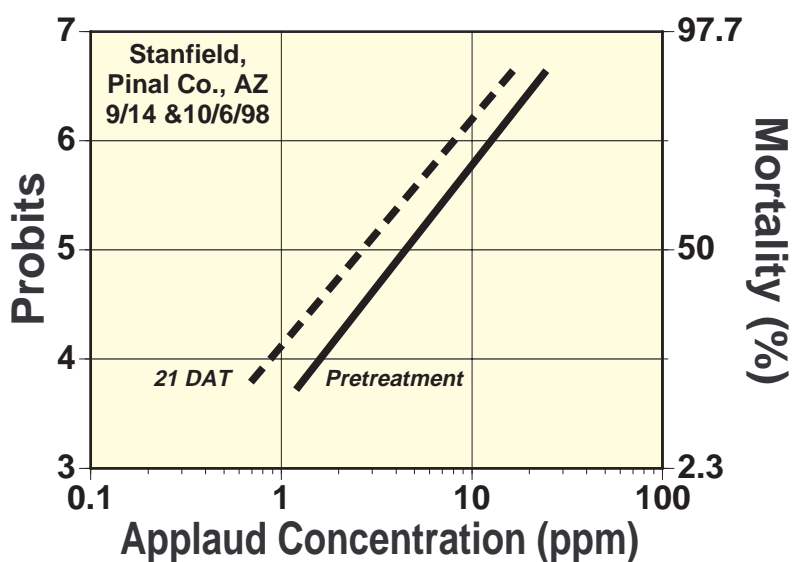


Figure 2. Probit lines for the 1998 Stanfield populations. The 21 DAT population was more susceptible to Applaud than the pretreatment population from the same site. DAT = days after treatment of the field with Applaud.

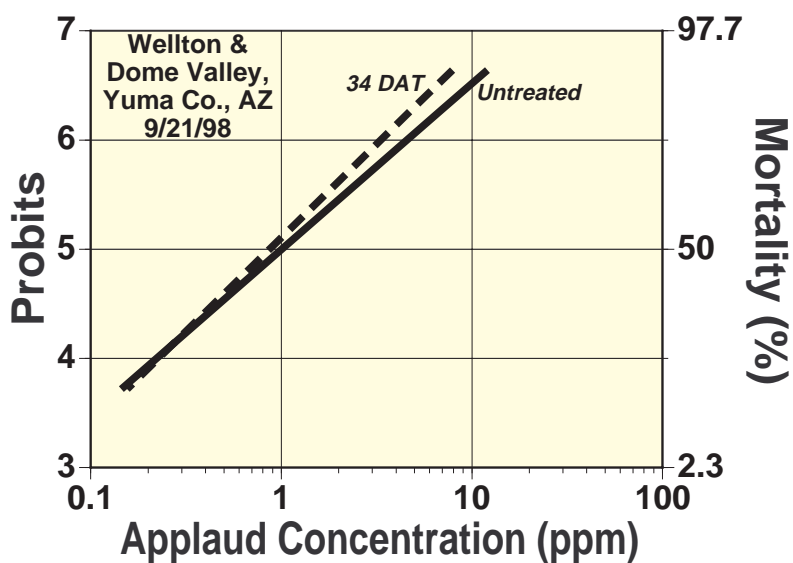


Figure 3. Probit lines for the 1998 Yuma County populations. The 34 DAT (Dome Valley) population was very similar in response to the untreated population (Wellton). DAT = days after treatment of the field with Applaud.

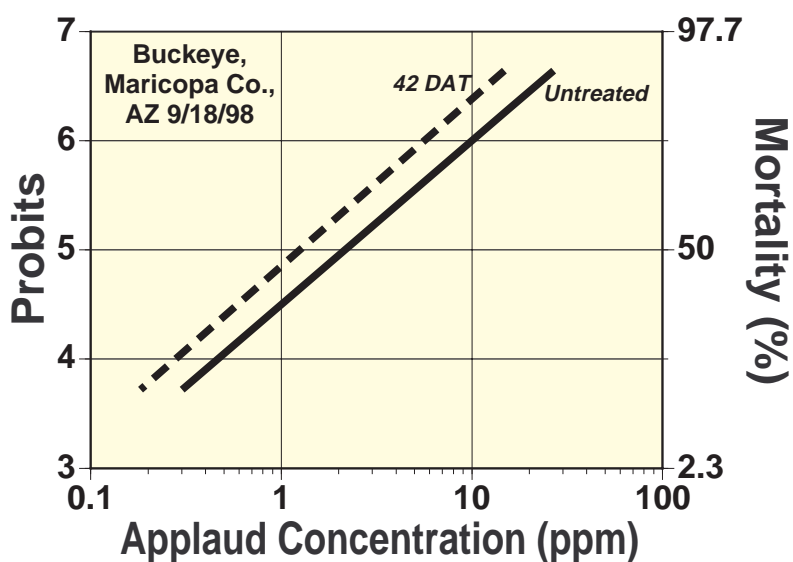


Figure 4. Probit lines for the 1998 Buckeye populations. The 42 DAT population was more susceptible to Applaud than the untreated population. DAT = days after treatment of the field with Applaud.

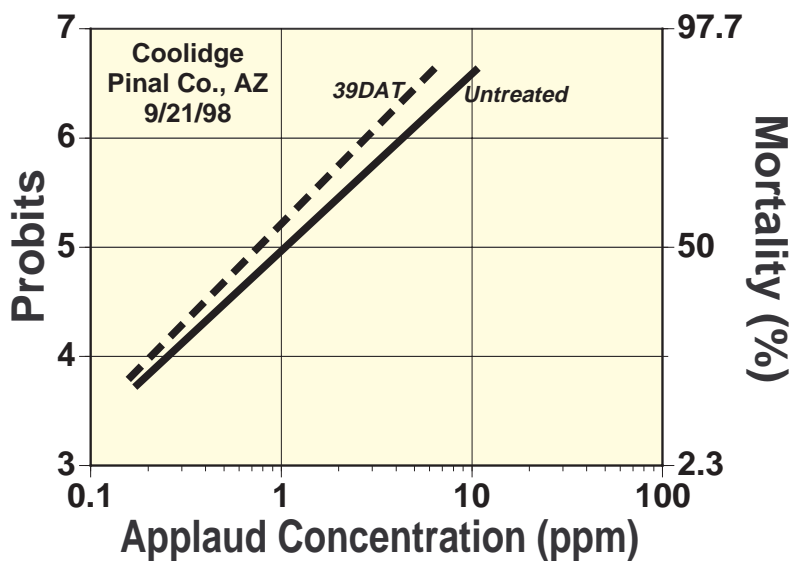


Figure 5. Probit lines for the 1998 Coolidge populations. The 39 DAT population was more susceptible to Applaud than the untreated population. DAT = days after treatment of the field with Applaud.

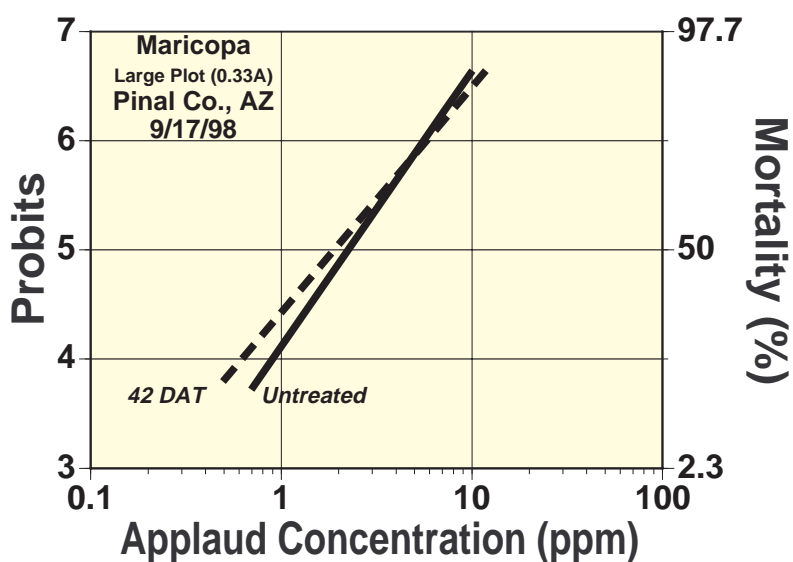


Figure 6. Probit lines for the 1998 Maricopa large plot populations. The 42 DAT population response was similar to the untreated population. DAT = days after treatment of the field with Applaud.

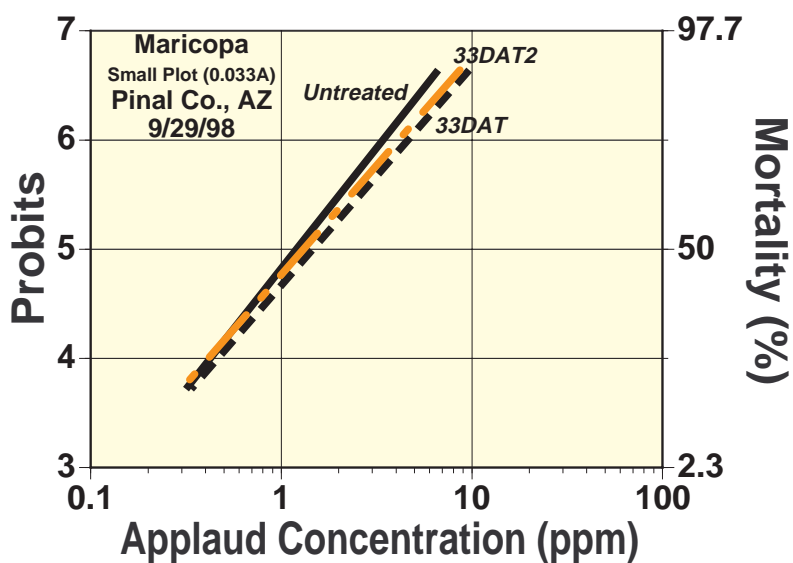


Figure 7. Probit lines for the 1998 Maricopa small plot populations. The 33 DAT population response was similar to the untreated population. Furthermore, a two-spray regime of Applaud+endosulfan did not significantly change the susceptibility of the whitefly population (33 DAT2). DAT = days after treatment of the field with Applaud.

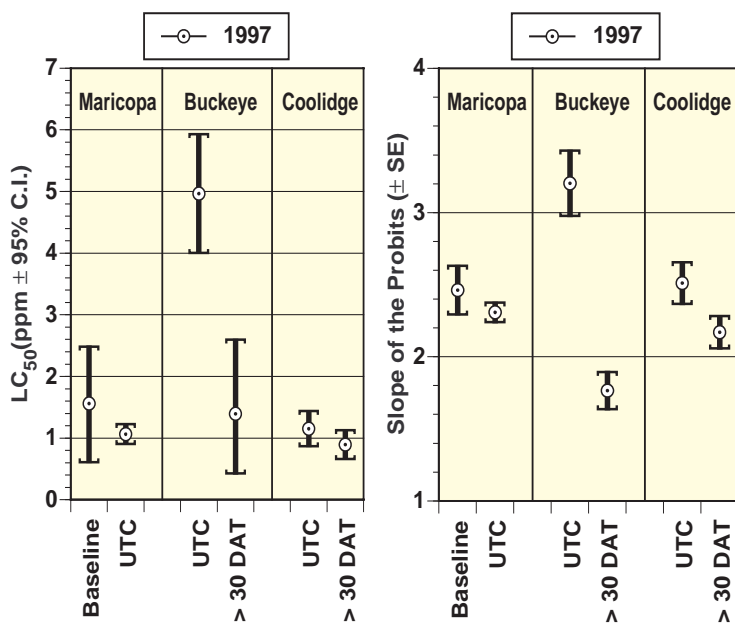


Figure 8. Probit parameters, LC_{50} (\pm 95% C.I.) (left) and slopes (\pm SE) (right), for Arizona populations in 1997. All populations were collected within a 15-day period starting in September. Field populations were collected from paired fields, one treated and one not treated ('UTC') with Applaud in 1997. The Maricopa sites were from untreated fields. DAT = days after treatment of the field with Applaud.

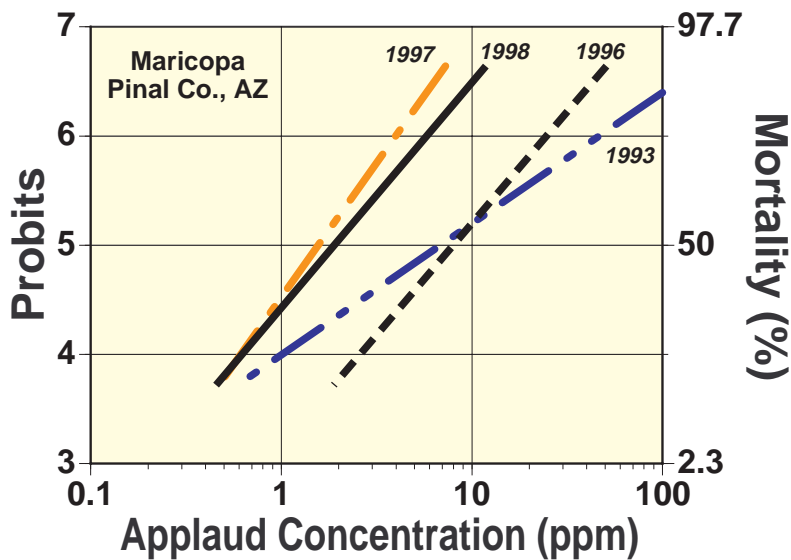


Figure 9. Probit lines for the 1993, 1996, 1997 Maricopa UTC and the 1998 Maricopa large plot treated (42 DAT) populations. The 1993 and 1996 bioassays were conducted under different conditions. The 1993 bioassays were conducted with a different formulation of Applaud (40SC) and a different wetting agent (Kinetic). Higher humidities were used in 1993 (60–70% RH) and 1996 (70–80%) than in 1997 or 1998 (40% RH). Nevertheless, even when comparing post-treated populations in 1998 with 1997 or earlier untreated populations, SWF susceptibilities have not declined.

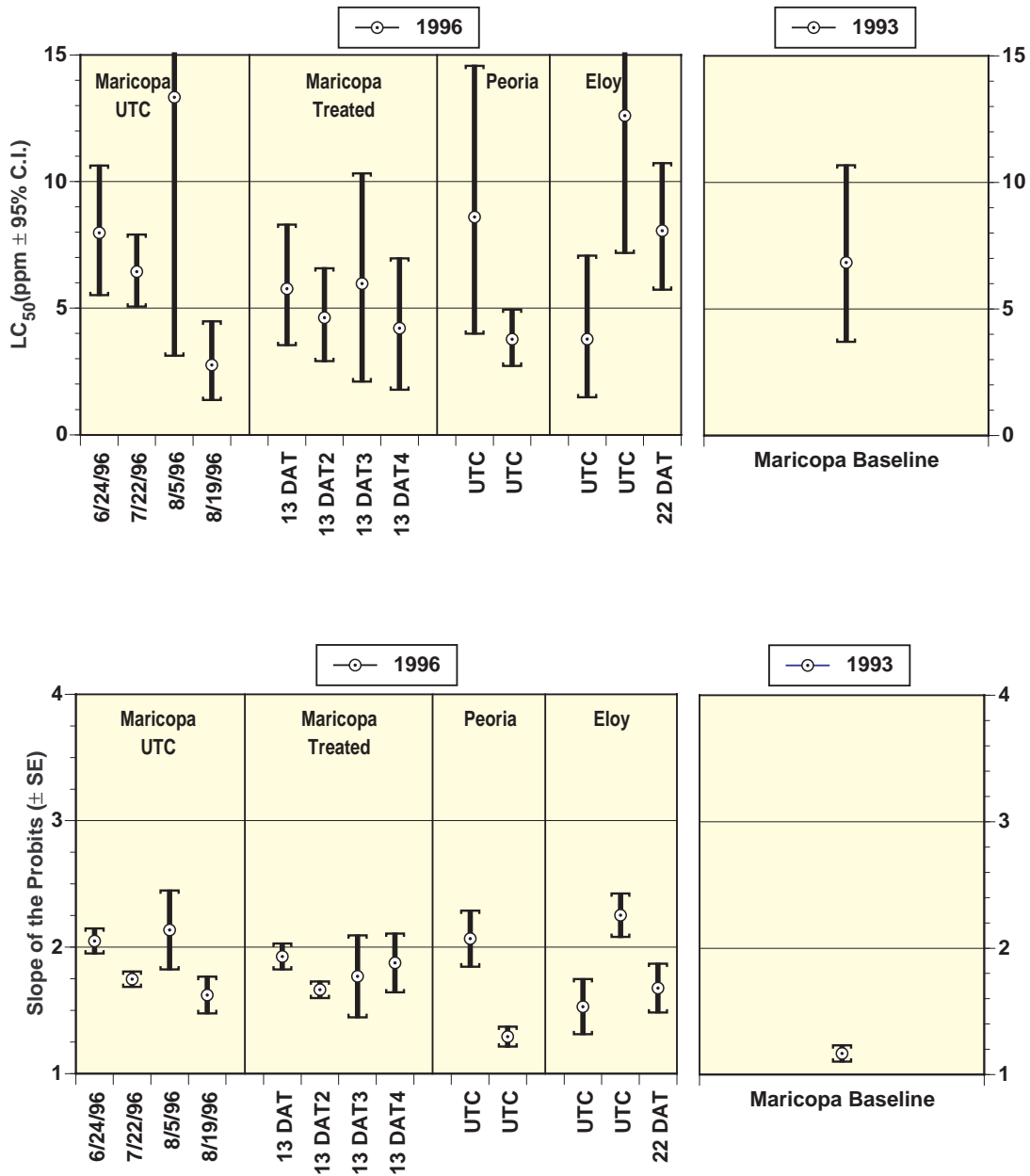


Figure 10. Probit parameters, LC₅₀ (± 95% C.I.) (above) and slopes (± SE) (below), for Arizona populations in 1996 (left) and 1993 (right). The Eloy populations were collected from paired fields, one treated and one not treated ('UTC') with Applaud in 1996. The Maricopa populations were from replicated untreated and treated plots. Populations were evaluated 13 days after 1, 2, 3 and 4 sprays of Applaud at the Maricopa treated site. No Applaud had been used in the U.S. prior to 1 July 1996. DAT = days after treatment of the field with Applaud.