Monitoring Changes in *Bemisia tabaci* (Hemiptera: Aleyrodidae) Susceptibility to Neonicotinoid Insecticides in Arizona and California

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ABSTRACT *Bemisia tabaci* (Gennadius) biotype B is a highly prolific and polyphagous whitefly that established in much of North America during the 1980s. Neonicotinoid insecticides have been fundamental in regaining control over outbreak populations of *B. tabaci*, but resistance threatens their sustainability. Susceptibility of *B. tabaci* in the southwestern United States to four neonicotinoid insecticides varied considerably across populations within each year over a 3 yr period. Using a variability ratio of highest LC_{50} to lowest LC_{50} in field-collected whitefly adults from Arizona and California, the ranges of LC_{50}s across all tests within compounds were highest to imidacloprid and lowest to thiamethoxam. Patterns of susceptibility were similar among all four neonicotinoid insecticides, but the greater variability in responses to imidacloprid and significantly higher LC_{50}s attained indicated higher resistance levels to imidacloprid in all field populations. Further evidence of differential toxicities of neonicotinoids was observed in multiple tests of dinotefuran against imidacloprid-resistant lab strains that yielded significant differences in the LC_{50}s of dinotefuran and imidacloprid in simultaneous bioassays. To test the possibility that resistance expression in field-collected insects was sometimes masked by stressful conditions, field strains cultured in a greenhouse without insecticide exposure produced significantly higher LC_{50}s to all neonicotinoids compared with LC_{50}s attained directly from the field. In harsh climates such as the American southwest, resistance expression in field-collected test insects may be strongly influenced by environmental stresses such as high temperatures, overcrowding, and declining host plant quality.

KEY WORDS systemic uptake bioassay, insecticide resistance, desert environment, host plant

Losses in agricultural production because of infestations of *Bemisia tabaci* (Gennadius) have increased the past 25 yr as virulent biotypes have been spread worldwide. The initial characterization of variant forms of *B. tabaci* as biotypes began when indigenous and invasive populations in North America were unambiguously identified and designated as biotypes A and B, respectively (Costa and Brown 1991). The practice of alphabetizing variants of *B. tabaci* that were conveniently called 'biotypes' continued as new variants were identified (Bedford et al. 1994), reaching a total of 24 with still others remaining unassigned (Perring 2001). The ongoing spread of biotype B to additional continents and ensuing destruction of crops was the catalyst that expanded awareness of *B. tabaci* as a global pest represented by geographically and genetically distinct variants (Brown et al. 1995, De Barro et al. 2011). Although the taxonomic and phylogenetic relationships remain in flux, the complex as a whole constitutes one of the most severe pests of agriculture worldwide. The extraordinarily adaptive and prolific nature of *B. tabaci* in toto often results in direct feeding damage to crops, fouling of crop commodities such as cotton by honeydew excreta, epidemics of viral diseases as a vector of over 110 viruses (Jones 2003), and induction of feeding disorders in plants such as squash silverleaf and tomato irregular ripening.

The B-biotype remains dominant, perhaps exclusive, in North America despite a subsequent widespread invasion by Q first detected in 2004 (McKenzie et al. 2009, Dennehy et al. 2010). The apparent failure of Q-type to establish in North America is noteworthy given that it was a case of extraordinary resistance identified in a resistance monitoring survey in Arizona that provided the essential clue and led to the discovery of the Q biotype in North America (Dennehy et al. 2010). The propensity for insecticide resistance to develop in *B. tabaci* has long been recognized and even suggested as a principal cause of outbreaks (Byrne et al. 1990). The incidence and diversity of resistance mechanisms in the outbreak-prone biotypes B and Q support this notion (Nauen et al. 2002, Rauch and Nauen 2003, Karunker et al. 2008), although intrinsic differences in resistance expression among biotypes are largely inferred because of differences in exposure histories. Nevertheless, varying degrees of insecticide resistance in biotype B compared with indigenous biotypes (Costa et al. 1993, Denholm et al. 1996) or to biotype Q (Horowitz et al. 2005, Wang et

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al. 2010) have been recorded and considered important to the relative success of either biotype.

Management limitations previously imposed by fewer modes of action for whitefly control 20 yr ago have been ameliorated by a dramatic expansion of new and effective insecticides. A shift to newer chemistry has been paramount to bringing more effective control of *B. tabaci* infestations (Palumbo et al. 2001, Ellsworth and Martinez–Carrillo 2001). In particular, neonicotinoid insecticides have played a leading role in combating *B. tabaci* in various cropping environments. Plant systemic characteristics of neonicotinoids have made possible a range of insecticide formulations that more effectively target *B. tabaci* and other pest species. Versatility in the timing of applications, whether at planting as a seed-coated or a soil-drench formulation to meet early pest pressure, or later during stand establishment and maturation as a soil-drench or foliar formulation, has contributed to the success of the neonicotinoids in combating *B. tabaci* infestations. Strong global demand for neonicotinoid insecticides can be measured in terms of market share that rose to 24% by 2008 (Nauen and Jeschke 2011).

Neonicotinoid insecticides have been instrumental in regaining control of outbreak populations of *B. tabaci.* The prolonged suppression of *B. tabaci* infestations in vegetable and melon crops treated with imidacloprid initiated a sustained recovery from devastating outbreaks that occurred in the southwestern United States in the early 1990s (Perring et al. 1991, 1993). Subsequent development and registration of thiamethoxam, acetamiprid, and dinotefuran provided additional choices of neonicotinoid insecticides on certain vegetable and melon crops and expanded preferential usage to other crops, notably cotton. Efforts to coordinate usage of nenicotinoid insecticides across crop commodities and minimize selection pressure were introduced in Arizona (Palumbo et al. 2003), but overall selection pressure remains elevated because of their effectiveness as a group against *B. tabaci* and the demand this creates in the pest management marketplace.

Maintaining efficacy of the neonicotinoids against *B. tabaci* has been a key goal of resistance management efforts in the United States (Dennehy and Williams 1997, Palumbo et al. 2001, Schuster et al. 2010). Monitoring responses of *B. tabaci* to neonicotinoids and other insecticides contributes essential information on susceptibility levels over time and provides a platform from which changes in susceptibility can be more readily interpreted. Detection of susceptibility changes to neonicotinoids provides an opportunity to make adjustments in a chemical control program that will mitigate further selection pressure, but also provides valuable insight into the dynamic nature of insecticide resistance in *B. tabaci* (Horowitz et al. 2004). Our goal with the current study was to further the understanding of resistance dynamics in the southwestern United States by monitoring the relative susceptibility of seasonally variable populations of *B. tabaci* to four neonicotinoid insecticides.

Materials and Methods

Bioassay Techniques. A systemic uptake bioassay was used in tests that compared all four neonicotinoids against *B. tabaci.* After comparing to a foliar bioassay, the systemic uptake bioassay was adopted for monitoring neonicotinoid insecticides against *B. tabaci* in the southwestern United States (Prabhaker et al. 2005) because it more closely approximates the exposure pattern that occurs in treated crops following translocation of mobile neonicotinoid insecticides from roots and stems to leaves fed upon by whiteflies. Because no soil interface is involved, all four compounds were used successfully in the systemic uptake bioassay. Moreover, comparisons between systemic uptake and foliar bioassays showed that systemic uptake bioassays were much more toxic to *B. tabaci* adults than foliar bioassays.

Systemic Bioassay. A standardized procedure was developed that used excised cotton leaves for the uptake of neonicotinoid insecticides before confining whitefly adults in clip cages for a 24 h feeding period. Robust comparisons of relative toxicities were made possible through simultaneous testing of all four neonicotinoids against field or greenhouse strains of *B. tabaci.* Fresh solutions of formulated insecticides were made up the first morning of the 2 d test. Six concentrations of each insecticide ranged according to time of year and perceived susceptibilities of test populations, but most commonly were spread from 100 to 0.32 μg/ml in a half-log series, although dropping as low as 0.032 μg/ml on occasion. Prepared solutions were transferred by pipette in 9 ml volumes to 1.59 by 7.62 cm (diameter by height) aqua piks equipped with rubber septa caps for holding leaf petioles snuggly while preventing evaporation from the reservoir. At least five replicates of each concentration for each test chemical were arranged in wooden racks with drilled holes for maintaining the tubular aqua piks. Cotton leaves from potted cotton plants were cut by razor blade at the juncture point between petiole and stem and the petiole immediately placed into aqua-piks to begin the uptake period. To minimize variation because of size or age, cotton leaves from only the first or second node of a 5–6 node plant were used. Weekly plantings of cotton were made in a greenhouse to ensure an abundant supply of test leaves.

Once all excised leaves were situated in racks to avoid overlap among leaves, racks were placed in an incubator equipped with high output fluorescent lights (14:10 diurnal cycle) and maintained at 24°C. Following a 24 h uptake period, a duplicate set of aqua piks was prepared to transfer the leaves from the chemical solutions into water only. By measuring the volume of chemical solution remaining in each aqua pik, the amount of solution that each test leaf had taken-up was determined. Whitefly adults collected from the field or greenhouse colony were transferred to a wooden box with solid, dark sides and a glass top from which active flyers could be easily aspirated. A single ventilated clip cage (3.2 by 1.2 cm d by h) was attached to each leaf to which 30 whitefly adults were

released to the abaxial side through a small port that was then corked. Whitefly adults quickly settled in their normal posture on the underside of leaves and commenced feeding. Once all whiteflies had been transferred, the racks holding the aqua piks were transferred into a growth chamber (28°C) and left undisturbed for 24 h before scoring mortality for each treatment and concentration. Test leaves were carefully removed from aqua piks followed by removal of clip cages and gently laid on the stage of a compound microscope to avoid disturbing live whitefly adults. Standing whiteflies were quickly tallied before proceeding to nonstanding whiteflies that were scored alive if any repetitive movement occurred. All results were subjected to probit analysis using PoloPlus (LeOra Software, CA), and LC_{50}s and 95% CIs were used to compare differences among whitefly sources and chemical treatments.

Foliar Bioassay. The setup for the foliar bioassay was similar to the systemic bioassay in that the same solutions were used, clip cages were attached to excised leaves in aqua piks held in wooden racks, and whitefly adults from the same sources as those used for systemic bioassays were aspirated and confined within the clip cages for a 24 h period. The difference was that solutions were applied to saturation as foliar sprays to leaf surfaces and allowed to dry within a fume hood for 2 h before attaching clip cages and adding whitefly adults. No adjuvant was added to the solutions because they were also used in systemic bioassays in cases where direct comparisons between the two bioassay methods were made.

Insect Strains. *Field Populations.* Collections of adult whiteflies were made from commercial cantaloupe and cotton fields in the Imperial Valley of California. In Arizona, collections were made at the University of Arizona's Yuma Agricultural Center and Maricopa Agricultural Center located in the southwestern and south–central parts of Arizona, respectively. Adult whiteflies were most often collected from crop or weed foliage into an inverted nylon stocking within the suction tube of a hand-held, battery-operated vacuum device. The stocking was removed every 30–60 s and everted to release captured whiteflies onto 3–5 potted cotton plants confined within a holding cage transported to the collection site. On other occasions, heavily infested leaves (>800 adults) on field plants could simply be clipped and placed within the holding cage. The presence of live cotton plants in the holding cage allowed whiteflies to settle and feed until they were returned to the lab. All test subjects were introduced to the bioassay either the same day they were collected in the field or the following day.

Lab Strains. Field-collected samples of *B. tabaci* adults used in bioassays were occasionally established as colonies maintained on cotton under cage and without insecticide exposure in greenhouses. Large (0.8 by 0.8 by 1.0 m) organdy screened colony cages with a hinged door and a reach-through sleeve were used to culture robust colonies on multiple cotton plants (6–12 leaf stage). The purpose was to track the responses of discrete genetic pools of *B. tabaci* through time to neonicotinoid insecticides and determine if the moderating conditions of greenhouse culturing affected their responses in bioassays.

In addition to field strains, an imidacloprid resistant-selected strain and laboratory reference strain were used in tests that compared bioassay techniques and to evaluate resistance stability in greenhouse colonies established from field strains. The GU-R strain originated on melon crops grown in Guatemala and was imported under permit in multiple shipments in 2000–2001 (Prabhaker et al. 2005). It was maintained under occasional pressure with imidacloprid in the greenhouse. The reference strain was collected on untreated cotton in 1998 and maintained in greenhouse colony cages without exposure (Prabhaker et al. 2005).

Insecticides. The following four neonicotinoid insecticides of formulated grade were provided by the respective manufacturing company: 1) acetamiprid (Intruder1 70% active ingredient [AI]) from DuPont, Wilmington, DE; 2) dinotefuran (Venom1 2 EC) from Valent, Walnut Creek, CA; 3) imidacloprid (Admire 2 F) from Bayer Ag, KS City, MO; and 4) thiamethoxam (Platinum 2 SC) from Syngenta, Oxnard, CA. Stock and serial dilutions for the formulated compounds were made with water on the day of tests for use in systemic bioassays.

Statistical Analysis. Results of the concentration-mortality experiments were analyzed using PoloPlus software to assess relative toxicities among neonicotinoid insecticides and allow comparison among populations. Differences in LC_{50} values were considered to be significant if there was no overlap in the 95% CL. Variability ratios for field populations were calculated by dividing the highest LC_{50} by the lowest LC_{50} obtained during a monitoring season. Further analysis of the concentration-mortality data were carried out by conducting analysis of variance (ANOVA) on the percent mortality response to each insecticide concentration used in the systemic uptake bioassays. Percent mortality data at each concentration was arcsine transformed and used as the response variable in a two-way ANOVA with neonicotinoid treatment and site-year as main effects. The site-year effect represented three discrete collections made in the Imperial Valley in 2004 as well as in Maricopa, AZ, in 2004 and in 2005. Significant F-values in the ANOVA for either of the main effects or their interaction were followed with a Tukey's honestly significant difference (HSD) means separation test. Graphical interpretation of the pattern of LC_{50}s from each site-year was done in JMP (Graph Builder, JMP 9.0.0, SAS Institute 2010) using a smoothing function featuring a cubic spline with a lambda of 0.05 and standardized X values.

Results

Bioassay Comparisons. Results of systemic uptake and foliar bioassays conducted simultaneously on field and laboratory strains showed large differences in relative toxicities between bioassay methods and among insecticides. In the sample of *B. tabaci* adults

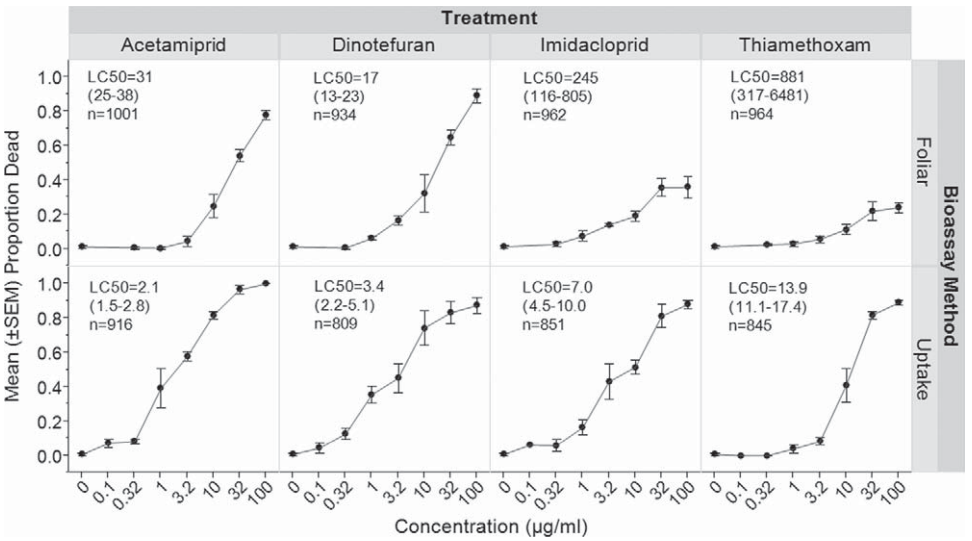

Fig. 1. Comparison of two bioassay methods and four neonicotinoid insecticides against a field population of *Bemisia tabaci* collected from cantaloupe in the Imperial Valley, CA, in 2005. Inset numerical figures within each chart panel represent the computed LC_{50}, 95% CLs (in parentheses), and number of insects tested for each insecticide and bioassay method. For foliar bioassay, $n = 5$ reps per dose; uptake bioassay, $n = 4$ reps.

from a springtime population in an Imperial Valley cantaloupe field in 2005, LC_{50}s in the systemic bioassay ranged from 2.1 (95% CIs = 1.5–2.5) for acetamiprid to 13.9 (11.1–17.1) for thiamethoxam, while the range of LC_{50}s in the foliar bioassay was from 17 (12.9–21.9) for dinotefuran to 880 (317–6481) for thiamethoxam (Fig. 1). For each insecticide, no overlap of 95% CIs between bioassay methods occurred, indicating that profiles of susceptibility generated for each insecticide were unique according to bioassay method. Higher LC_{50}s for acetamiprid and dinotefuran in the foliar bioassay resulted largely from reduced mortalities at the lowest concentrations (0.1–3.2), whereas relatively lower mortalities at all concentrations were observed for imidacloprid and thiamethoxam (Fig. 1).

Similar differences in magnitude between foliar and systemic bioassays were observed in a comparative test of dinotefuran and imidacloprid against the imidacloprid-pressured resistant strain GU-R (Prabhaker et al. 2005). Dinotefuran was relatively effective against the GU-R strain in both types of bioassays with a resistance factor (LC_{50imid}/LC_{50dino}) of 41 in the systemic uptake bioassay and 85 in the foliar leaf-dip bioassay (Fig. 2).

Field Monitoring. An initial comparison of neonicotinoid insecticides against field-collected samples of *B. tabaci* was made in 2003 using imidacloprid and thiamethoxam in a series of four tests (Fig. 3). The first sample of whiteflies collected from late-season cotton on 18 August was especially susceptible to both insecticides. The lowest concentration of imidacloprid (0.1 μg/ml) killed 67% of test insects ($n = 139$) compared with just 3.5% for thiamethoxam at the same concentration ($n = 223$). The extreme mortality precluded computation of an LC_{50} for imidacloprid, but for thiamethoxam the LC_{50} of 0.318 confirmed a highly susceptible population. Over the next 7 wk, susceptibility levels declined as the next three samples were collected from cantaloupe fields. By 5 October, only 3% of test insects at the 0.1 μg/ml concentration of imidacloprid were killed ($n = 175$). The decline in susceptibility was even greater at other concentrations of imidacloprid, for example, at 0.47 μg/ml in which 97% mortality occurred on 18 August ($n = 177$) compared with 24% on 5 October ($n = 177$). Comparison of the lowest to the highest LC_{50}s for each compound yielded a variability factor of 24 for imidacloprid and 10.2 for thiamethoxam (Fig. 3).

Monitoring susceptibilities of *B. tabaci* to all four neonicotinoid insecticides continued in 2004–2005 with series of bioassays from collections made in both California and Arizona (Fig. 4). Although relative susceptibility to individual insecticides varied through the season, the overall profiles generated within a season were similar for all four insecticides. For example, the V-shaped profile seen for results of acetamiprid, dinotefuran, and thiamethoxam bioassays on *B. tabaci* from the Imperial Valley, CA, reflected a pattern of modest tolerance in the spring, acute susceptibility during midsummer, followed by increasing tolerance again in the fall. The one departure from this pattern occurred with imidacloprid because of an elevated LC_{50} (31.3) on 1 July for recently immigrated whiteflies into a mid-season cotton field after dispersing from adjacent imidacloprid-treated cantaloupe fields (Fig. 4a). A few weeks later, LC_{50}s for whiteflies collected from the annual weed Wright's ground cherry (*Physalis acutifolia* (Miers)) plummeted to levels of around 0.2 μg/ml for imidacloprid, acetamiprid, and dinotefuran.

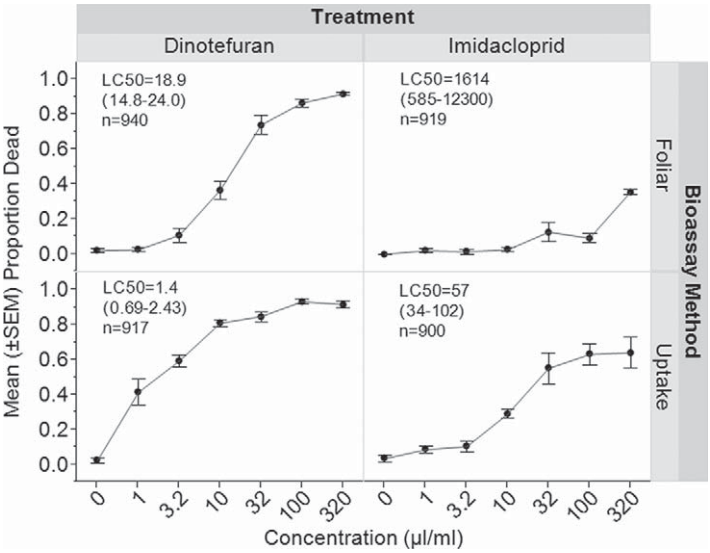

Fig. 2. Comparison of two bioassay methods and two neonicotinoid insecticides against an imidacloprid-resistant lab strain (GU-R) of *Bemisia tabaci*. Numerical figures in each chart panel represent the computed LC_{50}, 95% CLs (in parentheses), and number of insects tested for each method and insecticide. For each bioassay, $n = 5$ reps per dose.

A later seasonal expansion of *B. tabaci* populations in Maricopa, AZ, coinciding with growth of the cotton crop resulted in a different susceptibility profile compared with the multicrop system in the Imperial Valley in 2004. The first bioassay date yielded the lowest LC_{50}s of this series for acetamiprid, imidacloprid, and thiamethoxam (Fig. 4a). An overall increase occurred thereafter but for a dip in mid-October that yielded the lowest LC_{50} of the season for dinotefuran. After this point, higher tolerances to all four neonicotinoids were observed for whiteflies sampled from broccoli. In particular, LC_{50}s of 89 and 111 μg/ml were recorded for imidacloprid on 24 November and 3 December 2004.

In 2005, susceptibility profiles were again similar among the four neonicotinoids, but departed from the previous year's Maricopa, AZ, profile. The deep V-shaped profiles seen for acetamiprid, dinotefuran, and imidacloprid were mainly because of the year's first collection of whiteflies on 13 July from a spring-planted watermelon crop (Fig. 4b). Elevated LC_{50}s were observed for all four insecticides, especially to imidacloprid with an LC_{50} of 183. One week later, whiteflies collected from cotton resulted in the lowest

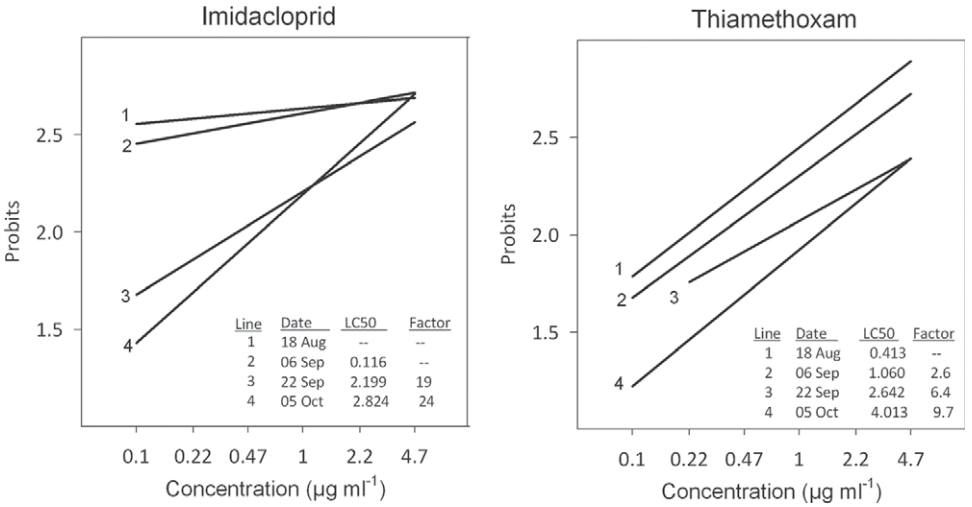

Fig. 3. Results (represented by log concentration-probit lines) of a series of systemic uptake bioassays conducted over a 7 wk period in 2003 in which LC_{50}s for each insecticide progressed to higher levels. Variability factors ($LC_{50higher}/LC_{50lower}$) computed at each interval (see tables within charts) showed a greater range of variability for imidacloprid than for thiamethoxam.

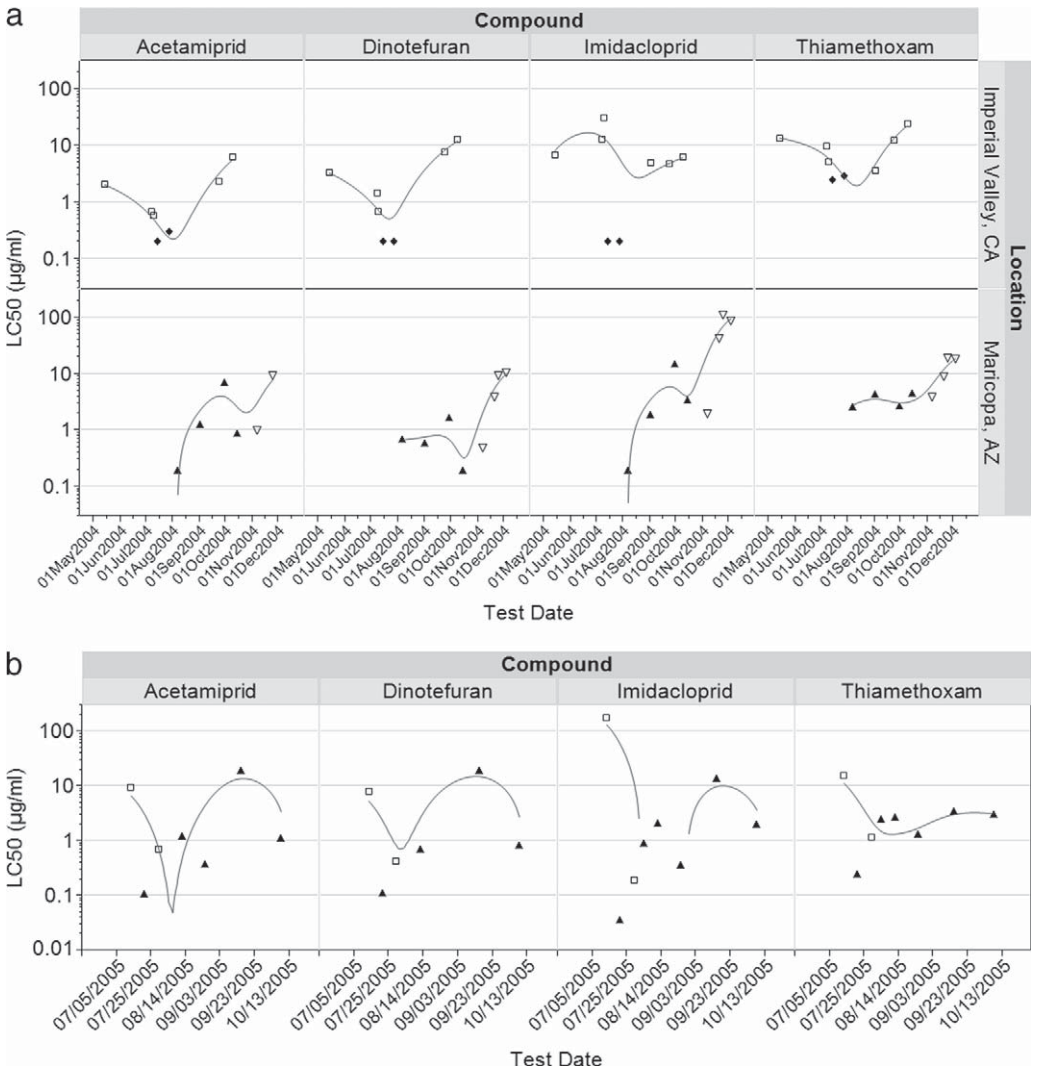

Fig. 4. Susceptibility profiles of *B. tabaci* from the Imperial Valley, CA, and Maricopa, AZ, to four neonicotinoid insecticides in (a) 2004, and (b) from Maricopa, AZ, in 2005. Marker symbols represent the different crops or weed species from which collections were made □ = melon, ▲ = cotton, ▽ = broccoli, ◆ = Wright's ground cherry).

LC_{50}s of the season for all four compounds. The following week's collection of whiteflies from a declining late-season cantaloupe crop on 29 July yielded relatively weak LC_{50}s to all four insecticides. By mid September, greater tolerance was observed to all four neonicotinoids, but then declined again to relative weakness with the season's last sample from cotton. No broccoli crops were available for sampling in the fall of 2005, and thus monitoring was discontinued.

The greatest variation in LC_{50}s as well as the highest LC_{50}s among the four neonicotinoids was seen for imidacloprid during 2004 and 2005 in both California and Arizona (Table 1). The maximum LC_{50} obtained during each of the three site-years varied by 5.8-fold for imidacloprid (183 ÷ 31.3), 3.1-fold for acetamiprid, and <2-fold for dinotefuran and thiamethoxam. The greatest variability between highest and lowest LC_{50}s within a site-year was observed in Maricopa, AZ, in 2005 for all four neonicotinoids.

Clear differences among compounds were seen in the proportion mortality at each concentration (Table 2). A highly significant F-value was obtained at all but the 0.1 µg/ml concentration. The mortality response for thiamethoxam was weakest at the lowest concentrations through the 3.2 µg/ml concentration, but did not differ statistically from acetamiprid or dinotefuran at the 32 and 100 µg/ml concentrations. Mean mortality at the lowest two concentrations of imidacloprid did not differ statistically from acetamiprid or dinotefuran, but was significantly lower at all higher concentrations. There was no significant statistical difference in mortality of whitefly test subjects between acetamiprid and dinotefuran at any concentration.

Table 1. Range of LC_{50}s and relative susceptibility factors for four neonicotinoid insecticides against adult *B. tabaci* from three site-years

Insecticide	Imperial Valley, CA (2004)			Maricopa, AZ (2004)			Maricopa, AZ (2005)		
	n	Range LC_{50} (μg/ml)[a]	Factor	*n*	Range LC_{50} (μg/ml)	Factor	*n*	Range LC_{50} (μg/ml)	Factor
Acetamiprid	7	0.2–6.5	32	6	0.2–9.6	48	7	0.11–20.1	183
Dinotefuran	7	0.2–13.1	65	8	0.2–10.5	52	6	0.11–19.3	168
Imidacloprid	8	0.2–31.3	156	8	0.2–111	555	8	0.10–183	1830
Thiamethoxam	8	0.2–25.1	125	8	2.6–19.6	7	8	0.25–15.7	63

[a] Variability ratios = highest LC_{50}/lowest LC_{50} for each compound across all bioassays reported in Fig. 4.

Greenhouse-Cultured Field Strains. A field strain of *B. tabaci* collected in Maricopa, AZ, from broccoli in December 2003 was retained as a greenhouse colony on cotton following an imidacloprid uptake bioassay that demonstrated reduced susceptibility based on an LC_{50} = 89 (Fig. 5a). Periodic bioassays thereafter showed increasing resistance to imidacloprid over the next 13 mo despite no insecticide exposure. This provided impetus to evaluate whether a highly susceptible field strain collected during summer would also express higher tolerance to imidacloprid once it was established as a greenhouse colony. A collection made 1 September 2004 from cotton in Maricopa, AZ, yielded an LC_{50} = 1.9 in an imidacloprid uptake bioassay (Fig. 5b). Left-over whiteflies not used in the bioassay were established as a greenhouse colony on cotton and tested periodically over the next several months. Although LC_{50}s obtained from the greenhouse cultured strain were significantly higher than the field LC_{50}, the level of resistance observed in this strain was much lower than the earlier cultured strain in Fig. 5a. A possible exception occurred in the 28 January bioassay as indicated by the flat response at higher concentrations in, but otherwise the mild resistance exhibited in this strain appeared less stable (Fig. 5b). Subsequent bioassays conducted on this strain in May and July saw a return to susceptibility with LC_{50}s of 22.8 and 3.3, respectively. In contrast to the variability observed in the field strains, bioassay responses of the laboratory reference strain remained relatively steady throughout the period from December 2003 to August 2005 with no more than a 6.4-fold variation in LC_{50}s within each set of data for the respective neonicotinoid insecticides.

Discussion

The mode of exposure of target insects to neonicotinoid insecticides can vary according to label specifications or by circumstance depending on crop stage, soil texture, and so forth. For example, acetamiprid is not labeled for soil application because of its rapid degradation by aerobic soil metabolism, whereas imidacloprid, thiamethoxam, and dinotefuran can be applied to the soil for systemic plant uptake or directly to foliage as spray applications. Application flexibility for most of the neonicotinoid insecticides enables them as a group to conform to a diversity of pest and crop management situations (Nauen and Jeschke 2011).

In terms of which method of application or exposure should be used in a resistance monitoring program, the choice of bioassay methodology has also proven flexible as both foliar (Nauen et al. 1998, Horowitz et al. 2004) and systemic uptake bioassays (Gorman et al. 2007, Schuster et al. 2010) have been used. In the southwestern United States, it was clear from the early years of imidacloprid use that soil applications were much more effective against *B. tabaci* than foliar sprays. The predominant application of imidacloprid in vegetable and other high value crops continues to be as a formulated material applied directly to the soil or through irrigation systems to the soil for systemic uptake by roots. To maintain continuity with earlier studies (Prabhaker et al. 1997, 2005), systemic uptake bioassays were used in this study to evaluate responses of field populations to imidacloprid and the other three neonicotinoids. The extremely high LC_{50}s observed with imidacloprid and thiamethoxam against a

Table 2. Analysis of variance on three data sets combined (Imperial Valley, CA, 2004; Maricopa, AZ 2004, 2005) of systemic uptake bioassay data according to dose with proportion mortality (arcsine transformed) as the response variable and treatment and location or season as main effects

	Concentration (μg/ml)						
	0.1	0.32	1.0	3.2	10	32	100
F-value	$F_{3,194} = 2.91$	$F_{3,363} = 18.1$	$F_{3,365} = 30.7$	$F_{3,364} = 23.3$	$F_{3,360} = 5.13$	$F_{3,338} = 6.9$	$F_{3,175} = 4.3$
Probability	$P = 0.0357$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0018$	$P = 0.0002$	$P = 0.0062$
Acetamiprid	0.101a	0.276a	0.527a	0.738a	0.834a	0.897a	0.926a
Dinotefuran	0.095ab	0.332a	0.555a	0.728a	0.833a	0.871a	0.929ab
Imidacloprid	0.131ab	0.277a	0.434b	0.621b	0.721b	0.802b	0.843b
Thiamethoxam	0.030b	0.074b	0.172c	0.459c	0.785ab	0.904a	0.937a

Post hoc analyses of treatment effects by Tukey's HSD test yielded significant differences among treatment mean mortalities indicated by different column letters.

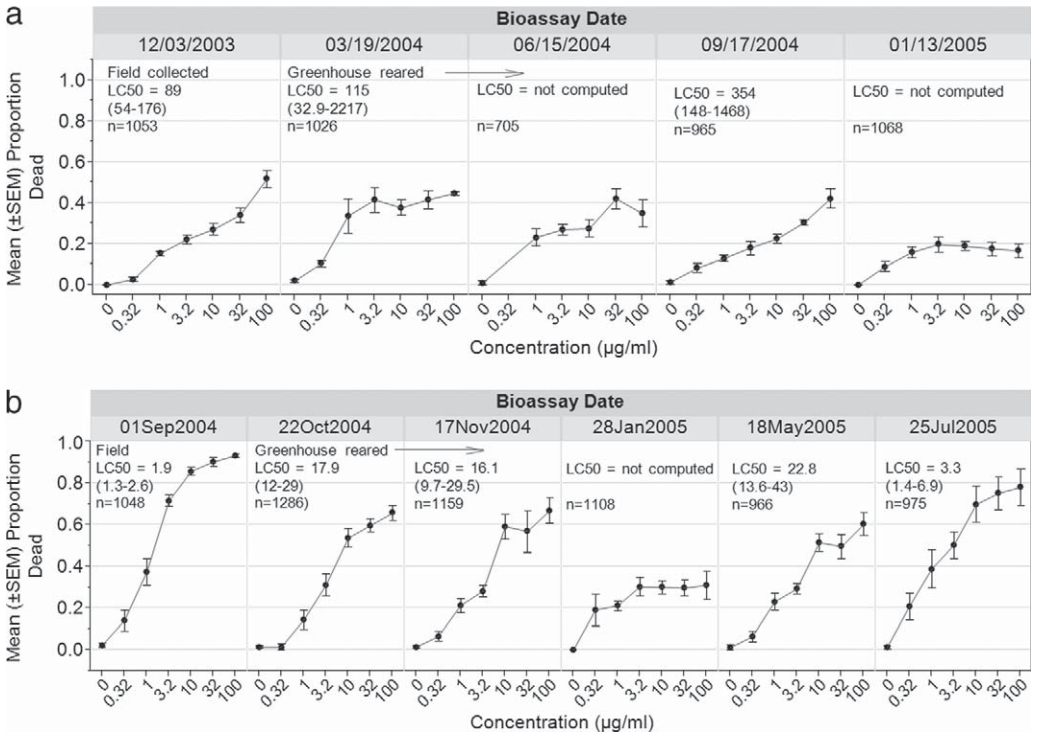

Fig. 5. Two series of charts showing imidacloprid bioassay results for field strains of *B. tabaci* collected in (a) December 2003 on broccoli and (b) September 2004 on cotton. Both strains were subsequently cultured on cotton as individual greenhouse colonies. Numerical figures in each chart panel represent the computed LC_{50} (except for those bioassay results that did not conform to the probit analysis model), 95% CLs (in parentheses), and number of insects tested for each method and insecticide. For each bioassay, $n = 5$ reps per dose.

field sample of whiteflies in the foliar bioassay (Fig. 1) indicated that flat responses across concentrations might represent a problem for a foliar bioassay when the goal is a full series of concentrations for generating probit statistics. In contrast, the uptake bioassay used on the same strain enabled a routine analysis that did not suggest anything extraordinary in terms of resistance to any of the four neonicotinoid insecticides. Similarly, the comparison of foliar and uptake bioassays on the imidacloprid-resistant GU-R strain again demonstrated that the anemic response across imidacloprid concentrations in the foliar bioassay could present a problem when the goal is to obtain an LC_{50} and other probit statistics (Fig. 2). However, both foliar and uptake bioassays worked well with dinotefuran against the field and GU-R strains, and for acetamiprid against the field strain. Thus, depending on the goals of the monitoring program and the characteristics of the target populations that will be subjected to monitoring, some version of a foliar bioassay may be suitable. In the current study, however, the comparatively flat responses of imidacloprid and thiamethoxam against the GU-R and field strains, respectively, in the foliar bioassay compared with uptake bioassay point to the more robust nature of the uptake bioassay for characterizing a wider range of responses of *B. tabaci* to neonicotinoid insecticides. The full toxicological potential of neonicotinoid insecticides is better expressed in systemic uptake bioassays where plant metabolism of active ingredients produces metabolites, some having toxicities on par with or even greater than parent compounds (Nauen et al. 1999).

In the desert agricultural regions of California and Arizona, deteriorating conditions in the cotton crop for a leaf-feeding herbivore are most likely to occur beginning in late July and thereafter as bolls are maturing, heat stress is occurring (Salvucci and Crafts–Brandner 2004, Pettigrew 2008) and pest infestations are increasing. The V-shape response profiles observed in the Imperial Valley 2004 and the Maricopa 2005 data sets show a consistency among the four neonicotinoids in overall susceptibility patterns of whiteflies. In both data sets, initial bioassays of whiteflies collected from melon crops were followed by collections made on a summer annual weed in the Imperial Valley or on cotton in Maricopa, AZ, that yield much lower LC_{50}s . The potential influence of highly stressful conditions on the performance of *B. tabaci* in insecticide bioassays cannot be discounted, especially in light of the minimal LC_{50}s that generally occurred during summer months for all four neonicotinoids. This is a phenomenon that has been observed previously in a resistance monitoring program for conventional insecticides conducted in the same region (Prabhaker et al. 1992, 1996).

The responses over multiple generations of two field strains of *B. tabaci* established in greenhouse cultures support the idea that stressful environments from which test insects are collected may at times mask the expression of resistant genotypes. Both the fall-collected strain from broccoli and the late-summer collected strain from cotton demonstrated higher resistance to imidacloprid after being cultured in the greenhouse. On some of the subsequent bioassay dates for each strain, essentially flat responses across concentrations precluded computation of LC_{50}s, but nevertheless point to even higher levels of imidacloprid resistance than indicated by the highest LC_{50} for each strain. This pattern of increasing resistance after being cultured without exposure to imidacloprid represents a marked departure from previous studies that have shown a decline in resistance. For example, Schuster et al. (2010) recorded a progressive decrease in thiamethoxam and imidacloprid resistance over a period of five and six generations, respectively, after culturing field strains collected from Florida tomato fields. Similarly, mild resistance to thiamethoxam and imidacloprid in a strain of *B. tabaci* from Mexico decreased after six generations in the absence of selection pressure (Gutierrez–Olivares et al. 2007). However, not all cases of neonicotinoid resistance in *B. tabaci* have diminished after prolonged culturing. Resistance to neonicotinoid insecticides in numerous Q-type strains has proven to be quite stable over time (Nauen et al. 2002, Rauch and Nauen 2003) with one Spanish strain (E99–2) showing no loss of resistance after 25 generations without selection pressure (Nauen et al. 2002). Another case involved a Q-type strain imported from Spain to California under quarantine permit that showed extreme resistance to four neonicotinoids after culturing for 18 mo without insecticide exposure (Prabhaker et al. 2005). Such examples are not well known for B-types, although in one case a modest 2.4-fold decrease (874- to 361-fold) in resistance to imidacloprid occurred with no selection pressure over a 6 mo period in a B-type strain from Israel (Rauch and Nauen 2003).

Multiple factors likely affect resistance expression in both field-collected and laboratory-reared insects. The pattern of higher susceptibilities to various insecticide treatments during summer months observed in the desert southwest over many years suggests an important environmental component that can act to mask resistance expression in a bioassay. The conditions of high heat and aridity, declining plant quality, and high dispersal rates may individually or together push large numbers of whiteflies to the very limits of survival during summer months. From the time they are collected in the field and subjected to a laboratory bioassay no >24 h later, the capacity of test whiteflies to resist toxicants has potentially been compromised by the stressful conditions under which they have existed. Relaxation of high-stressed conditions may help to unmask the resistance traits and enable their expression in the present or subsequent generations.

The wide-ranging bioassay responses to the four neonicotinoids observed in this study underscore the dynamic nature of resistance, or more specifically resistance expression. Although *B. tabaci* populations in the desert southwest may indeed be resistant to imidacloprid and other neonicotinoids, how effectively that resistance is expressed in the field or laboratory may belie their underlying genetic capacity to resist these toxicants. The possibility that extrinsic factors influence resistance expression may help to explain why resistance is not simply progressive in nature, always building to higher levels, but in fact recedes as commonly as it advances, irrespective of insecticidal selective forces. Natural mortality factors acting nonselectively across genotypes may neutralize selective advantages accrued to insecticide-resistant genotypes and perhaps enable a resurgence of susceptible genotypes, especially if fitness costs are associated with resistant genotypes. This would tend to dilute the resistance gene pool and manifest as higher susceptibility levels, but only until the next imidacloprid-treated crop reselected for resistant genotypes during a season when environmental resistance was reduced.

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