Research Article

Received: 30 September 2013 Revised: 10 December 2013 Accepted article published: 24 December 2013 Published online in Wiley Online Library: 3 February 2014

(wileyonlinelibrary.com) DOI 10.1002/ps.3717

Comparative susceptibility of *Bemisia tabaci* to imidacloprid in field- and laboratory-based bioassays

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Abstract

BACKGROUND: *Bemisia tabaci* biotype B is a resistance-prone pest of protected and open agriculture. Systemic uptake bioassays used in resistance monitoring programs have provided important information on susceptibility to neonicotinoid insecticides, but have remained decoupled from field performance. Simultaneous bioassays conducted in field and laboratory settings were compared and related to concentrations of imidacloprid in plant tissue for clearer interpretation of resistance monitoring data.

RESULTS: Mean mortalities of adult whiteflies confined on cantaloupe leaves field-treated with three rates of imidacloprid did not exceed 40% in two trials. In contrast, laboratory bioassays conducted on different subsets of the same whitefly populations yielded concentration–response curves suggestive of susceptibility to imidacloprid in five populations (LC_{50} values from 1.02 to 6.4) relative to a sixth population ($LC_{50} = 13.8$). In the field, densities of eggs and nymphs were significantly lower on the imidacloprid-treated cantaloupes compared with the untreated control, but the margin of control was greater in 2006 than in 2007. The potential impact of imidacloprid on whitefly eggs was explored in a greenhouse test that showed egg mortality occurring in both early (one-day-old) and late (three-day-old) eggs on cotton leaves systemically treated with imidacloprid. Quantification of imidacloprid residues in cotton leaves used routinely in systemic uptake bioassays revealed concentrations that greatly exceeded concentrations found in the field-treated cantaloupe leaves, at least at the three highest solution concentrations used for uptake.

CONCLUSION: Systemic uptake bioassays have been widely used for monitoring *B. tabaci* resistance to imidacloprid, but without knowledge of imidacloprid concentrations that occur in test leaves relative to field concentrations. Higher mortality observed in systemic uptake bioassays relative to field-treated cantaloupes in this study suggests that field rates of imidacloprid are only partially effective against *B. tabaci* adults, in contrast to systemic uptake bioassays that showed susceptibility to imidacloprid. The discrepancy between field- and laboratory-based mortalities is probably due to extraordinarily high concentrations of imidacloprid that can occur in leaves of systemic uptake bioassays, potentially skewing perception of susceptibility to imidacloprid.

Keywords: systemic uptake bioassay; insecticide resistance; ELISA test; neonicotinoid; whiteflies

1 INTRODUCTION

Bemisia tabaci biotype B has been the principal pest of vegetable and field crops for many years in the American southwest and is also considered to be one of the most serious agricultural and horticultural pests worldwide.[1] Devastating outbreaks in the 1990s in California, Texas and Arizona resulted in lost farm revenues in excess of hundreds of millions of dollars.[2,3] Whitefly pest pressure during this period proved to be unrelenting as high population numbers were sustained on sequentially grown crops in spite of frequent insecticide applications. The commercial introduction of imidacloprid in North America in 1993 offered a distinct, underexploited mode of action with little or no cross-resistance to older insecticide classes.[4] It soon became the foundation of a chemical management program for *B. tabaci* that continued to diversify over time with the incorporation of new modes of action. One of the outcomes of greater chemical diversity was a return to susceptibility against certain pyrethroid and organophosphate insecticides that had been intensively used prior to the advent of newer insecticides, in effect restoring treatment options that had been nullified by intensive resistance.[5]

In spite of vast improvements in chemical control options over the past 20 years, the continuous use of imidacloprid in the southwestern United States has created intensive selection pressure on *B. tabaci* populations. High percentages of fall/winter vegetables and spring melons have been treated continually with imidacloprid from the time it was registered, a practice that has been exacerbated by lower prices on a proliferation of generic imidacloprid products since the compound came off patent in 2005. Another factor contributing to selection pressure has been the late-season dispersal of *B. tabaci* populations

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from summer field crops and ornamentals and their subsequent concentration on imidacloprid-treated vegetable fields during the cooler fall months. Coming out of winter, spring cantaloupes planted from January through March represent the most heavily imidacloprid-treated crop, with >90% of the acreage often treated during the spring season (http://calpip.cdpr.ca.gov/main.cfm). Successive treatments in fall/winter vegetables and spring melons during times of seasonally declining or expanding populations, respectively, prolong the exposure period and increase selection pressure of imidacloprid on *B. tabaci* populations, thus raising concerns over resistance development.

Heavy dependence on imidacloprid and other neonicotinoid insecticides for whitefly control has led to a growing number of resistance cases. The earliest example was from the Almeria region of Spain,[6–8] a place of intensive year-round vegetable production under more than 30 000 ha of protected agriculture. A prevalence of plant viral diseases in this region, including tomato yellow leaf curl virus (TYLCV) and cucumber vein-yellowing virus (CVYV), contributed to lower action thresholds, while a concomitant increase in chemical treatments exacerbated the resistance problem.[4] Additional cases have been reported from the Mediterranean basin, including high levels of resistance to thiamethoxam in Israel[9,10] and to imidacloprid in Crete[11,12] and Cyprus.[13] Oxidative metabolism of neonicotinoid compounds through enhanced expression of various cytochrome P450 genes, *CYP6CM1* in particular, has been implicated as a primary mechanism of imidacloprid resistance in several instances.[14–16] Additional P450s have been identified in resistant strains from China, including overexpression of *CYP6CX1* in a thiamethoxam-resistant strain compared with a susceptible strain.[17,18]

Biochemical or molecular assays that test for increased titers of detoxification enzymes or enhanced expression of detoxification genes are useful in identifying and characterizing resistance that is due to a specific metabolic enzyme[19] or target site such as insensitive AchE-R.[20–22] However, such capability arises only after thorough research to establish a causal relationship between the biochemical or molecular process and the phenotypic expression of resistance. Even then, monitoring data obtained by using a biochemical procedure must be understood to represent only the specific mechanism for which it is designed and not an overall appraisal of other potential resistance mechanisms in an organism. On this account, standardized toxicological bioassays that minimize variability of test conditions remain the most comprehensive measure of the genetic capacity of a strain or population to resist a toxicant. Bioassay techniques need not simulate field exposure in order to detect and document susceptibility differences among strains, but some validation to actual field performance is desirable.[23]

Systemic uptake bioassays have long been used for monitoring *B. tabaci* resistance to imidacloprid, but with little attention given towards relating exposure levels in bioassays to those that occur in the field. Developing a relationship between exposures encountered in the field to those in the laboratory is perhaps more critical for a systemically mobile compound such as imidacloprid than for a foliarly applied compound. Foliar insecticide residues deposited on leaves in a laboratory bioassay should closely approximate field residues so long as similar concentrations and volumes are used for spray deposition in both situations. In a systemic uptake bioassay, however, concentrations of solutions prepared for uptake by detached leaf petioles or plantlet stems are specific to the solution only and do not represent concentrations of active ingredients within plant tissues fed upon by test insects. Although consistent concentration responses are readily attained in systemic uptake bioassays, inferences that can be extended to exposure concentrations in bioassay test plants or field-treated plants are limited. The present study was initiated to evaluate the performance of imidacloprid against field populations of *Bemisia tabaci* using systemic uptake bioassays in the laboratory and simultaneously on field-treated cantaloupes using *in situ* bioassays. In addition, control exerted on *B. tabaci* immature stages on the field-treated cantaloupes relative to adult mortality in bioassays was studied to gain a fuller understanding of the toxicity spectrum of imidacloprid.

2 MATERIALS AND METHODS

2.1 Bioassay methods

2.1.1 *Field bioassay*

Small field plots (9.1 × 2 m) consisting of two rows (2 m centers) of drip-irrigated cantaloupes (cv. 'Topmark') were established at the University of Arizona Maricopa Agricultural Center in early September of 2006 and again in 2007. A randomized complete block design consisted of three Admire®Pro™ treatments (0.21, 0.315, 0.42 kg AI ha^{-1}) and an untreated control that were assigned randomly within each of four blocks. The 0.315 kg AI ha^{-1} rate is most commonly applied at planting to cantaloupes grown in the southwestern United States, with the lowest rate often applied in split applications, i.e. at planting and at the 4–6-leaf stage as a side-dress or through a drip-irrigation system. Formerly, the highest rate (0.42 kg AI ha^{-1}) was rarely applied owing to high costs, but it is now more commonly used with the advent of generic imidacloprid products. At the cotyledon stage, cantaloupe plants were thinned to 28 cm between plants prior to applying Admire Pro through a modular drip-irrigation system. Polyethylene drip tape with emitters spaced every 25 cm was laid over the tops of beds and then connected to a fertilizer pump that injected prescribed amounts of Admire Pro at a 1:100 mixing ratio with water. The modular drip system was configured to the layout of each treatment within the four blocks so that all four replications were treated with a single application.

Field-based bioassays were conducted on young cantaloupes at the two-true-leaf stage (21 September 2006, 14 September 2007) and later on mature vines (>10 mainstem leaves, 6 and 12 October 2006, 3 October 2007). Within each year, different collections of *B. tabaci* adults from various sources were assayed in the same plots but on different dates. For each plant, field-collected *B. tabaci* adults from various sources were confined by a clip cage[24] to the abaxial side of a leaf. The number of clip cages per treatment varied between 8 and 12 per treatment each date, depending on the availability of adults from field collections. Thirty adults were introduced into individual clip cages that were shaded from direct sunlight by a paper plate attached to a wooden stake over each caged leaf. Adult mortality was scored at 48 h after finding that insufficient mortality was produced at 24 h. During adult mortality assessments, caged leaves were slowly rotated so that the abaxial side faced up and live whiteflies were not disturbed as clip cages were carefully removed. Counts of live and dead adults on the leaf surface were made with the assistance of magnifying goggles. Each clip cage was then examined to count any dead adults dislodged from the leaf surface.

2.1.2 *Field monitoring of* B. tabaci infestations

In addition to conducting multiple short-term field bioassays, natural infestations on cantaloupes in the field bioassay plots were

monitored weekly for 6 weeks (21 September through 26 October 2006; 4 September through 10 October 2007). Leaf discs (2.5 cm^2) were punched from the fifth-node leaves from branch terminals. On younger plants with branches of $\leq$5 leaves, leaf punches were taken from the leaf at the first or second mainstem node. Six leaf discs from each plot were collected for a total of 24 discs per treatment each week, from which *B. tabaci* eggs and small and large nymphs were counted in the laboratory.

2.1.3 *Systemic bioassay*

Cotton leaves detached from greenhouse-grown plants were used for the uptake of imidacloprid prior to confining whitefly adults for an extended feeding period. Weekly plantings of cotton were grown to the 5–6-true-leaf stage and then moved into a room adjacent to the greenhouse the night before starting an uptake bioassay. Cotton leaves were detached from plants the next morning with a razor blade at the petiole axil from the first or second node to minimize variation in leaf size and age. Leaf petioles were immediately immersed in 10 mL aqua-piks equipped with a rubber septum cap for holding the leaf petioles while preventing evaporation from the reservoir. Each aqua-pik was filled with 9 mL of solution from one of 6–7 solution concentrations prepared the same day, plus the untreated control (water). Five replicates at each concentration for each test chemical were established with aqua-piks evenly spaced in wooden racks to allow uniform light exposure to each leaf. Carrying out this procedure away from direct sunlight avoided water-stressed test leaves and enabled good uptake of solutions across all concentrations.

Following a 24 h uptake period in a growth chamber at 26 °C and a 14:10 L:D cycle, a duplicate set of aqua-piks was prepared for transfer of the leaves from the chemical solutions into water only. The remaining solution from each aqua-pik was measured to determine the volume taken up by test leaves. A single clip cage was attached to each leaf to confine 30 whitefly adults aspirated from a caged cotton plant and released into each clip cage through an access port. Once all whiteflies were transferred, the racks holding the aqua-piks were transferred into a growth chamber (26 °C) and left undisturbed for 24 h prior to scoring mortality for each treatment and concentration. The criterion for mortality was complete absence of movement; moribund subjects were scored alive.

2.1.4 *Whitefly egg and immature bioassay*

A stand-alone greenhouse (floor dimensions 2.74 × 3.66 m) was equipped with an insect-proof screened cage surrounding the evaporative cooler and intake vents to ensure production of cotton plants completely free of all background infestations of *B. tabaci*. Cotton seeds were germinated and planted within this greenhouse, and 240 plants were grown to the 5–6-true-leaf stage. The first-node leaf of each plant was infested with 25 adults per clip cage. Adults used in clip cages came from a field population collected six weeks earlier from cotton in the Imperial Valley, California, in June 2006 and maintained for two generations on cotton within a greenhouse colony cage. After transferring adults to clip cages, the cotton plants were moved to a separate insect-proof greenhouse for 24 h to allow oviposition. Clip cages were removed the next day to vacuum all adults from infested leaves prior to returning the plants to the original clean greenhouse. Each first-node leaf on all 240 plants was now infested with a synchronized cohort of *B. tabaci* F_1 eggs. As this generation of synchronized eggs advanced to adults over the next 20 days, subsets of plants were pulled from the greenhouse for use in systemic uptake bioassays on whitefly developmental stages, including one- and three-day-old eggs and first, second, third and fourth instars. The first-node leaves containing the desired stage were detached and used in each bioassay as previously described. Each stage was tested by the same imidacloprid concentration series of 0.1, 0.32, 1.0, 3.2, 10.0 and 32.0 μg mL^{-1}, as well as by an untreated control (water). Six replications (leaves) at each concentration were used for the one- and three-day-old eggs and for first- and second-instar nymphs, and five replications per concentration for third- and fourth-instar nymphs. Adults of the F_1 generation were collected from cotton plants in a separate colony established at the same time as the clip cages were infested. After parental adults had been given a 24 h oviposition period, they were removed to allow the synchronous cohort of eggs to hatch and develop into 3–4-day-old adults which were used in the F_1 adult bioassay. Egg mortality was scored on the basis of failure to hatch after 8 days. Nymph mortality was determined after 48 h, based on partial or complete desiccation of the insect (i.e. raised margins or total detachment from the leaf respectively).

2.2 Insect strains

Collections of adult whiteflies used in the field and systemic uptake bioassays were made in 2006 from production cantaloupe fields in the Imperial Valley, California (21 and 22 September and 6 October), a cotton field near Maricopa, Arizona (22 September), and from a selection of residential ornamental plants in Phoenix, Arizona. In 2007, adult whiteflies were collected from cantaloupe field plots at the Maricopa Ag Center (14 September), landscape ornamentals in Phoenix, Arizona (14 September, 4 October), and Imperial Valley cantaloupes (4 October). Adult whiteflies for these tests were aspirated from the plants with a battery-powered suction device and released onto potted cotton plants confined within a transfer cage. All test subjects were used in a systemic uptake bioassay, field bioassay, or both, within 24–48 h of collection.

2.3 Imidacloprid in leaves

In 2007, concentrations of imidacloprid were measured in leaf disc samples collected from the field bioassays in cantaloupes and from cotton leaves used in systemic uptake bioassays. Leaf discs were punched immediately after completing bioassays from where clip cages containing whiteflies had been attached to leaf blades. After storing cantaloupe tissue samples in a −80 °C freezer, contents were extracted by shaking tissue samples in 75% methanol (2 mL) for 2 h. A commercially available ELISA kit (EP 006; Envirologix®, Portland ME, http://envirologix.com/) was used according to the manufacturer's instructions to quantify titers of imidacloprid in the respective tissue sample after methanol extraction and dilution in water (≥40-fold) to bring imidacloprid concentrations (ng mL^{-1}) into the range covered by the standard curve.

2.4 Statistical analysis

Probit statistics (LC_{50}, 95% confidence interval and slope) were calculated for the concentration–response data from systemic uptake bioassays using PoloPlus (Le Ora Software, Petaluma, CA). Statistical differences in LC_{50} values among field populations tested in systemic uptake bioassays or among stages in the whitefly egg and immature study were based on non-overlap of 95% CIs. A chi-square goodness-of-fit test evaluated the fit of the toxicity data to the probit model.

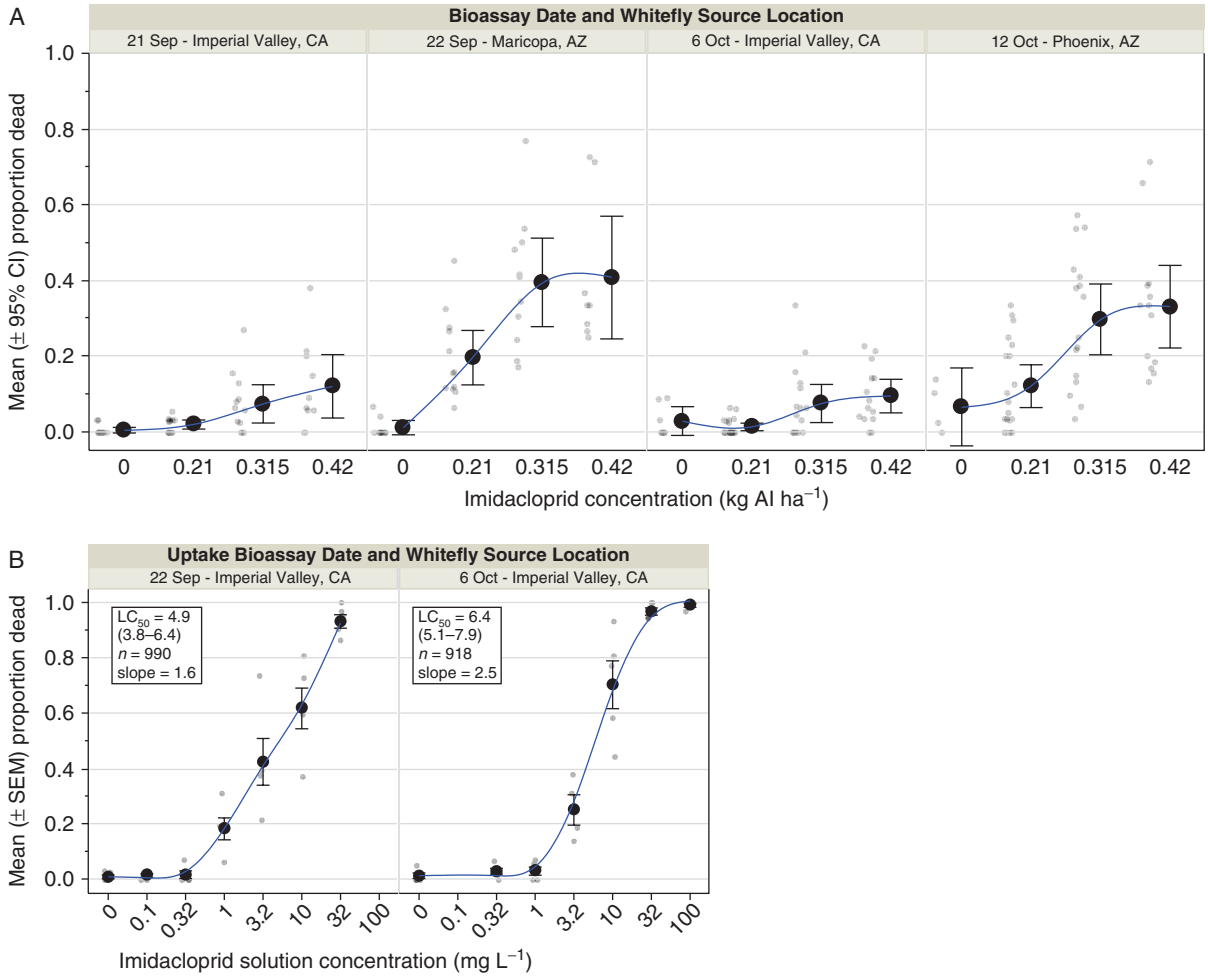

Figure 1. Field bioassays (A) of four different populations of *B. tabaci* conducted on cantaloupe treated with three rates of imidacloprid in 2006. Light-shaded points within each panel represent mortality on individual leaves; mean mortalities are represented by dark-shaded points (±95% CI). Systemic uptake bioassays (B) of two of the *B. tabaci* populations that were also assayed in the field.

The respective impacts of three different rates of imidacloprid on densities of whitefly eggs and immatures on cantaloupes in the field were evaluated by repeated-measures multivariate analysis of variance using Pillai's trace (MANOVA platform, JMP® 10.0, 2010; SAS Institute, Cary, NC) after a $\log_{10} x + 1$ transformation of the count data. Where the overall MANOVA was significant, further comparisons among treatments were made using contrasts.

3 RESULTS

3.1 Field and laboratory bioassays

Mean adult whitefly mortality from field-based bioassays of four *B. tabaci* populations on cantaloupe in 2006 (Fig. 1a) did not exceed 40% at the highest application rate or during the early-production season (September) when imidacloprid concentrations should be at near-peak levels. Differences in susceptibility to imidacloprid were apparent, especially between the Imperial Valley (assayed 21 September) and Maricopa (assayed 22 September) populations. Only slight increases in mortality with higher application rates were observed for all four populations. In the laboratory bioassays, mortality increased progressively with imidacloprid concentration for the Maricopa (22 September) and Imperial Valley (6 October) populations. Probit analyses yielded relatively low LC_{50} values for both populations (Fig. 1b).

A similar pattern of low mortality across application concentrations was observed in 2007 field bioassays (Fig. 2a). The highest mean mortality again was only 40%. This level of mortality was observed at the highest rate of imidacloprid in the 14 September assay of adult whiteflies collected from residential ornamentals in Phoenix, Arizona. Observed mortalities on other dates or for other whitefly populations were generally lower than 40%. Mortalities in the laboratory uptake bioassays for the same populations yielded relatively low LC_{50} values for the 4 October populations (Fig. 2b). However, the Maricopa whitefly population tested in the field on 14 September proved less susceptible to imidacloprid in both laboratory and field bioassays compared with the Phoenix population on the same date. Mortalities for the Maricopa population in the uptake bioassay were similar to those for the other three populations at the lowest three concentrations of imidacloprid, but were lower than for the other populations at concentrations of >3.2 mg L^{-1}.

3.2 Imidacloprid impact on eggs and immatures

Intense immigration pressure by dispersing *B. tabaci* occurred each year in the cantaloupe field plots. The highest egg counts in 2006 were recorded on the first sample date at a time of heavy adult crowding on young cantaloupe plants. Increases in plant biomass over subsequent weeks tended to ease adult

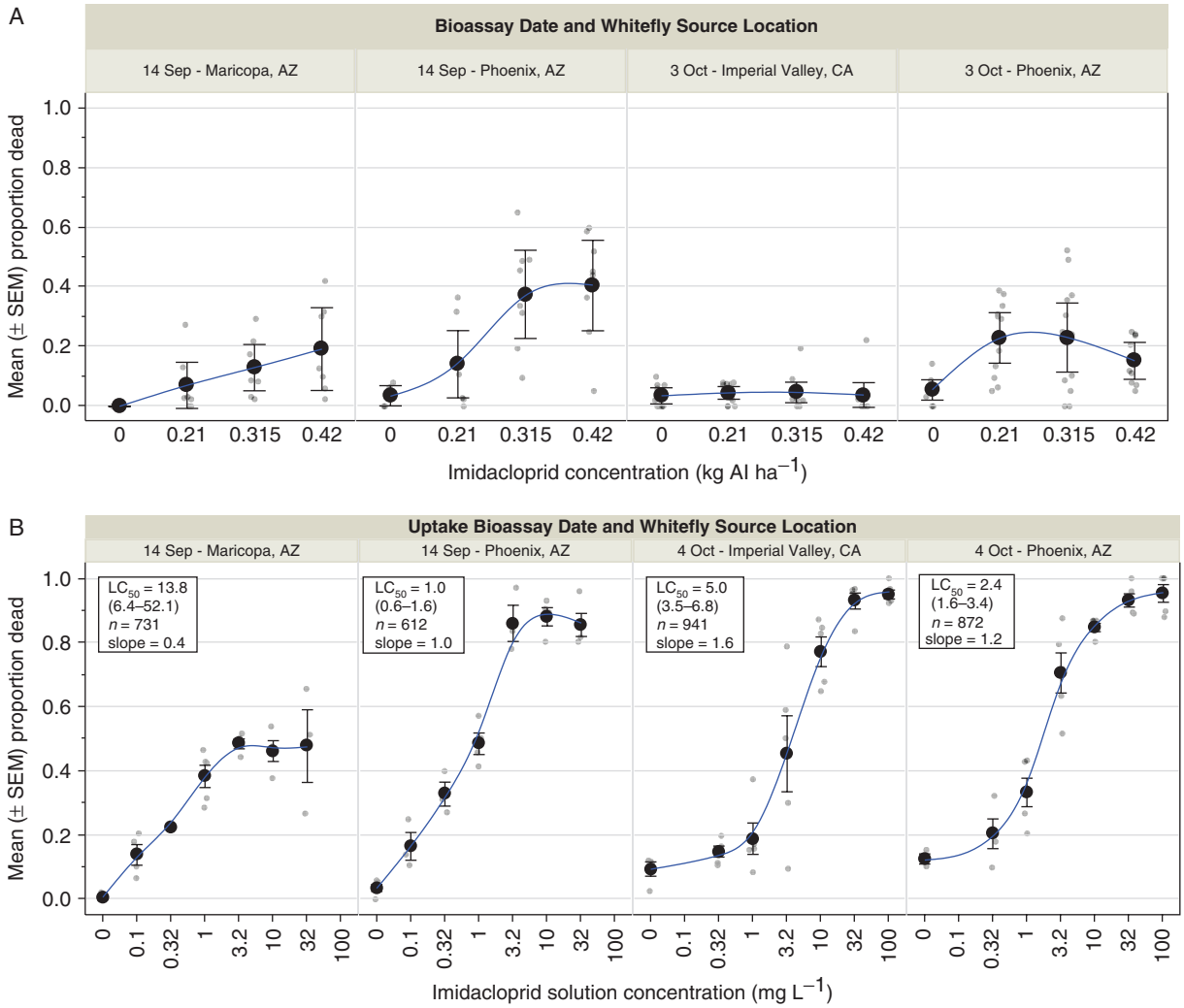

Figure 2. Field bioassays (A) of four different populations of *B. tabaci* conducted on cantaloupe treated with three rates of imidacloprid in 2007. Light-shaded points within each panel represent mortality on individual leaves; mean mortalities are represented by dark-shaded points (±95% CI). Systemic uptake bioassays (B) of the same four *B. tabaci* populations that were also assayed in the field.

crowding, which was reflected in lower egg and small-nymph counts through the remainder of the evaluation period (Fig. 3). Although lower densities were observed in the imidacloprid-treated cantaloupes relative to the untreated control, there were no significant differences among time × treatment interactions in densities of eggs ($P > 0.6$) or small nymphs ($P > 0.24$). Large-nymph densities, however, were significantly lower in all three imidacloprid treatments compared with the untreated control ($F_{9,21} = 2.67$, $P = 0.03$). In addition, large nymphs were significantly lower in the 0.42 kg ha^{-1} treatment compared with the 0.21 kg ha^{-1} treatment ($F_{3,5} = 6.89$, $P = 0.03$). In 2007, season-long densities evaluated by the time × treatment interaction were significantly different for all three imidacloprid treatments relative to the untreated control for eggs ($F_{15,15} = 7.58$, $P = 0.0002$) and small nymphs ($F_{15,15} = 3.01$, $P = 0.02$), but not for large nymphs ($F_{6,14} = 2.18$, $P = 0.11$). Individual contrasts among imidacloprid treatments indicated that the highest field rate of imidacloprid had significantly fewer eggs than the lowest rate ($F_{5,3} = 13.48$, $P = 0.03$). The sharp decline in egg densities in the untreated control on the last two sampling dates of 2007 corresponded to deterioration of cantaloupe vines owing to *B. tabaci* infestation that did not occur in any of the imidacloprid-treated plots (Fig. 4). Densities of small nymphs in 2007 were significantly higher at the 0.21 kg AI ha^{-1} treatment compared with the two higher treatment rates of imidacloprid, but no significant differences in densities occurred between the 0.315 and the 0.42 kg AI ha^{-1} rates.

All stages of *B. tabaci* originating from cotton in the Imperial Valley were susceptible to imidacloprid in the systemic uptake bioassay (Table 1). No significant mortality differences were recorded between one- and three-day-old eggs, nor between eggs and third-instar nymphs. First- and second-instar nymphs were significantly more susceptible to imidacloprid than any other stage (Table 1). The high degree of heterogeneity evident in the data for each whitefly stage resulted in consistently high chi-square values, which indicated poor fit to the probit model.

3.3 Imidacloprid in bioassay leaves

Imidacloprid concentrations in cantaloupe leaves used in field bioassays in 2007 reached a maximum mean concentration of 265 ± 60 ng mL^{-1} in the 0.42 kg AI ha^{-1} treatment on 7 September, 1 week after application. Mean concentrations showed a declining trend over the next 2 weeks, but with considerable variation

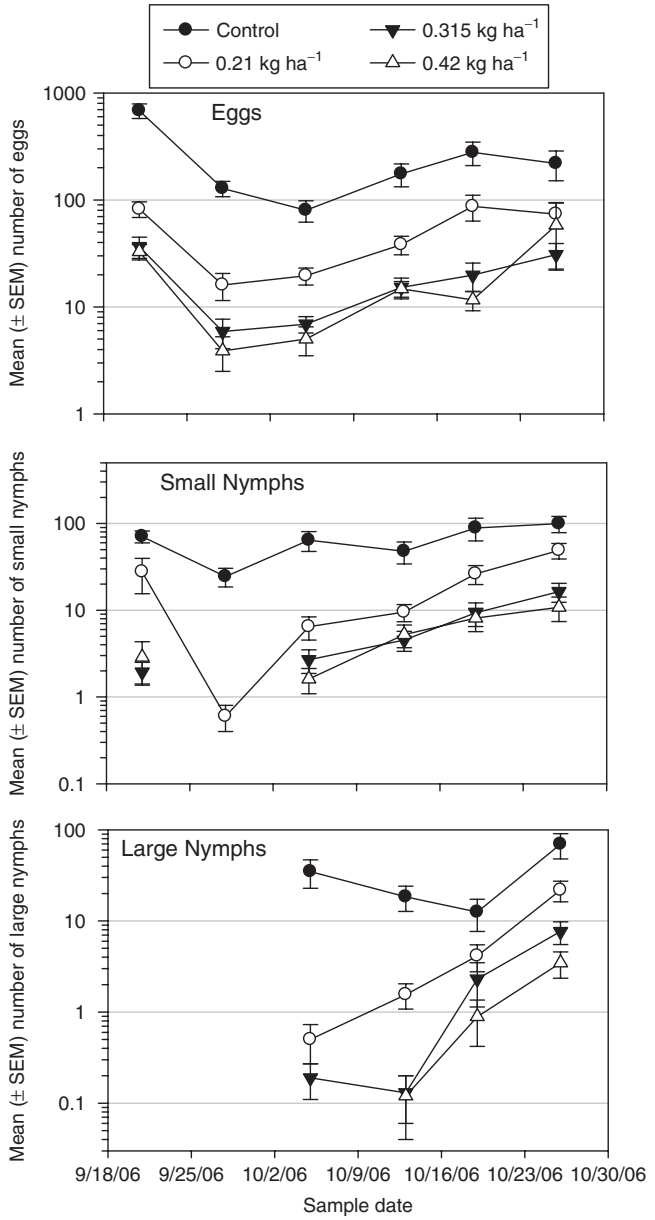

Figure 3. Mean (± SEM) number of *B. tabaci* eggs, small nymphs (first and second instars) and large nymphs (third and fourth instars) from field surveys of imidacloprid-treated cantaloupes at Maricopa, Arizona, 2006.

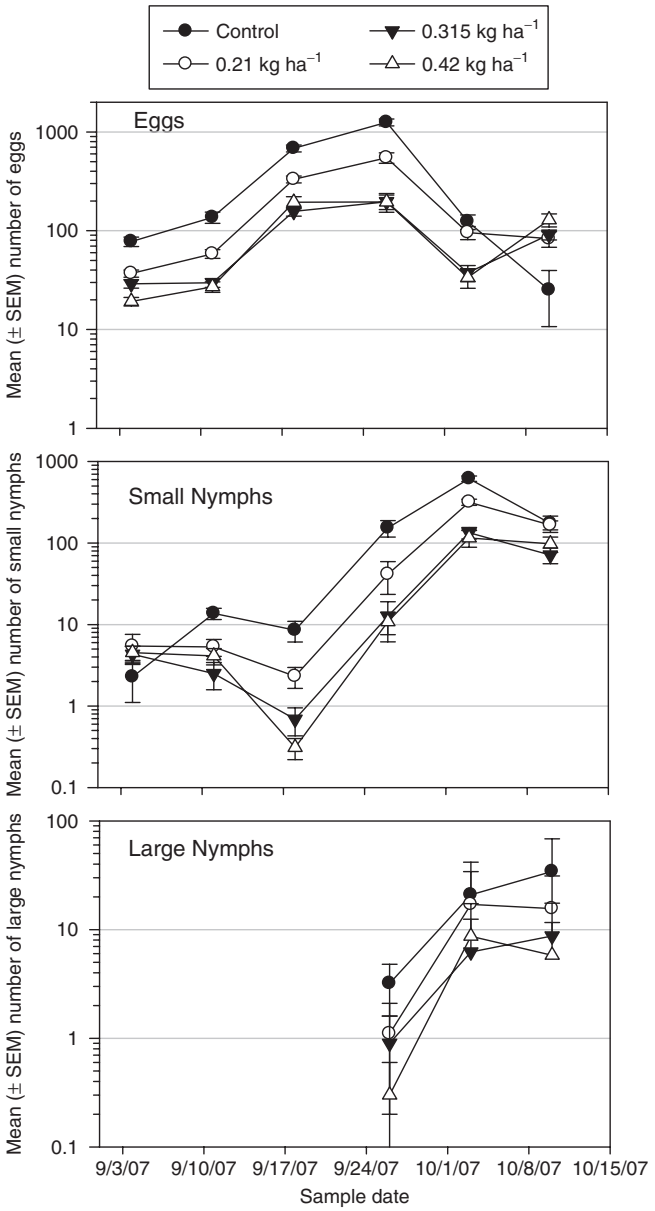

Figure 4. Mean (± SEM) number of *B. tabaci* eggs, small nymphs (first and second instars) and large nymphs (third and fourth instars) from field surveys of imidacloprid-treated cantaloupes at Maricopa, Arizona, 2007.

Table 1. Probit statistics derived from a series of imidacloprid systemic uptake bioassays performed on each life stage of a population of *B. tabaci* collected from cotton, Imperial Valley, California, 2006. Significant differences between LC_{50} values are indicated by different lower-case letters

Stage	Age	*n*	Slope (± SE)	LC_{50} (95% CI)	χ^2	df
Egg	1 day	1865	2.44(±0.14)	4.1 a (3.3–4.9)	95.7	33
	3 days	1169	1.97(±0.12)	2.9 a (2.3–3.7)	89.6	33
Nymph	First instar	1014	1.64(±0.09)	0.79 b (0.55–1.09)	140.9	34
	Second instar	1252	1.30(±0.056)	1.01 b (0.79–1.29)	76.2	34
	Third instar	1479	1.18(±0.05)	2.66 a (1.99–3.54)	107.8	34
	Fourth instar[a]	1211	—	—	59.6	34
Adult	F_1, 3 days	830	0.64(±0.06)	3.2 a (1.4–6.5)	109.3	27

[a] No probit statistics owing to non-conforming data.

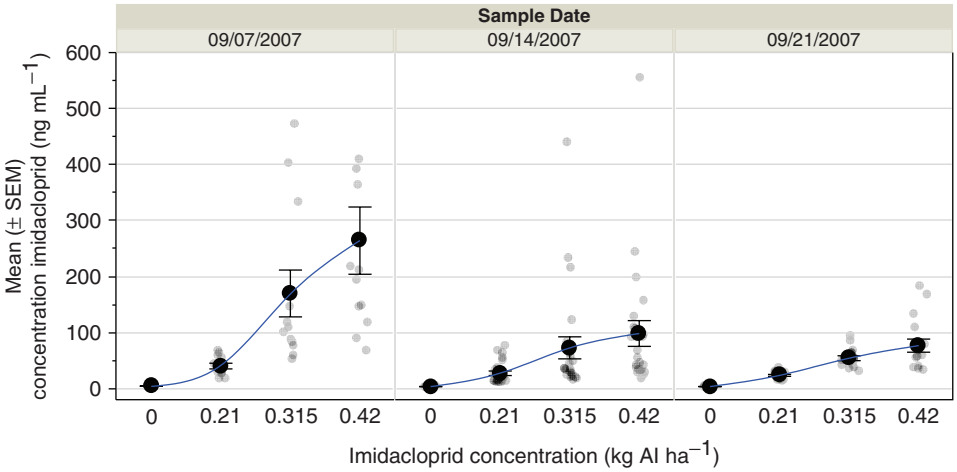

Figure 5. Mean (± SEM) concentrations of imidacloprid in methanol extracts from cantaloupe leaf discs sampled from field-grown cantaloupes, Maricopa, Arizona, 2007.

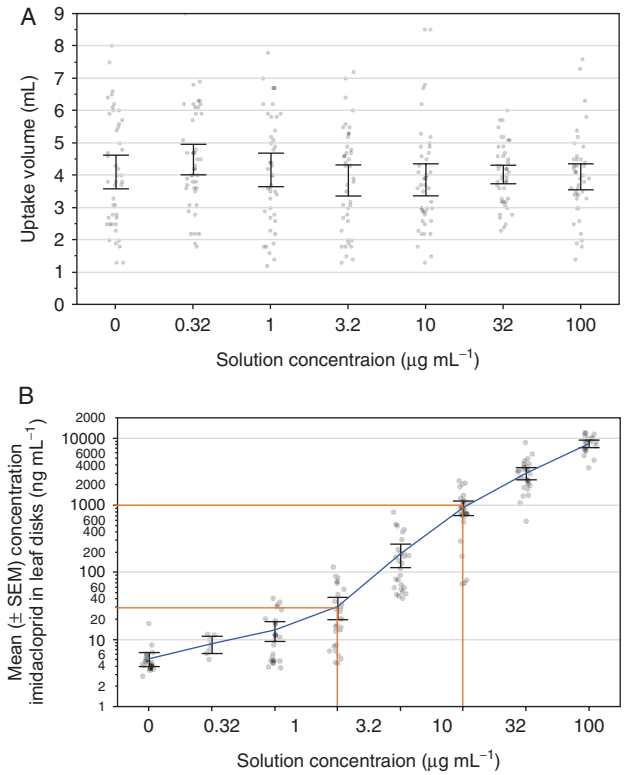

Figure 6. Mean (± SEM) uptake volumes (A) from cotton leaves used in systemic uptake bioassays of imidacloprid. Light-shaded points represent uptake volumes for individual leaves. Imidacloprid concentrations (B) in methanol extracts from leaves of five of the eight uptake bioassays. Guide lines drawn at 1 and 10 µg mL^{-1} indicate the approximate range of imidacloprid concentrations found in methanol extracts from field-grown cantaloupes.

among leaves in each treatment (Fig. 5). Similarly, a high degree of variation was observed in the systemic uptake bioassays, both in the volume of solution taken up by individual leaves (Fig. 6a) and in the concentration of imidacloprid in leaf discs (Fig. 6b). The range of concentrations measured in cantaloupe leaves from the field (Fig. 5) was well below the mean concentrations of imidacloprid in cotton leaves immersed in the 10, 32 and 100 µg mL^{-1} solution concentrations (Fig. 6b).

4 DISCUSSION

Systemic uptake bioassays have been used in numerous programs for monitoring resistance to imidacloprid,[6,25–27] but always with the understanding that the mortality response was based on solution rather than exposure concentrations. This has not been an impediment to monitoring changes in susceptibility, so long as a consistent response in a reference population was obtained that could serve as a baseline for evaluating potential shifts in wild-type populations.[6] A different approach has been to establish a response profile by evaluating numerous wild-type populations so that deviations from a typical response would signal a potential resistance problem. Investigations conducted in the southwestern United States have relied upon the profiling approach, but have failed to detect sustained shifts toward higher resistance.[25,28,29]

A major problem with the systemic uptake bioassay has been the uncertainty of not knowing the concentration of an insecticide to which a test insect has been exposed. This has led some investigators to abandon the systemic uptake bioassay for a foliar bioassay, but in the process sacrificing ingestion of the insecticide as the more customary and toxic mode of exposure relative to contact.[29] The ELISA kit assay for imidacloprid has enabled sensitive measurement of imidacloprid concentrations in leaf discs punched from bioassay leaves, as well as leaves sampled from crops in the field such as the cantaloupe in this study. The comparison of imidacloprid concentrations in the cotton leaves from the systemic uptake bioassay and cantaloupe leaves from the field showed differences greater than 35-fold at the highest concentrations for each bioassay. Differences of this magnitude help to explain why the mean mortality response in the field bioassays did not exceed 40% after a 48 h exposure, but exceeded 95% after 24 h in all but one systemic uptake bioassay. However, it is the occasional systemic uptake bioassay in which the mortality response plateaus (e.g. Fig. 2b, 14 September, Maricopa, Arizona) that occasionally raises concerns about imidacloprid resistance. Although high concentrations of imidacloprid are routinely detected in the leaf discs punched from leaves used in the systemic uptake bioassay, there is no supporting data

regarding the concentration of imidacloprid within the phloem tissue from which whiteflies feed.

Although no trend was associated with mean uptake volumes in cotton leaves across all solution concentrations, individual leaves varied tenfold or greater in concentration of imidacloprid within solution concentrations from 0.32 to 32 $\mu g\ mL^{-1}$. The variation in uptake and resulting concentration of imidacloprid to which whiteflies are exposed during a systemic uptake bioassay may introduce heterogeneity into the data which diminishes the precision with which the LC_{50} values can be estimated. In addition to the inherent weakness of associating solution concentrations used in systemic uptake bioassays to mortality responses of a test population, heterogeneous data caused by variability in solution uptake may result in high chi-square values that would indicate a poor fit of the data to the probit model.[30]

In spite of evidence that *B. tabaci* adults from field populations in Arizona and California can survive concentrations of imidacloprid that occur in field-treated crops, imidacloprid is still effective at suppressing *B. tabaci* populations in the field. Infestations of immature *B. tabaci* on the field-grown cantaloupes were maintained well below the untreated control even though adult mortality was minimal in the bioassays. This may have been due to differences between immature and adult *B. tabaci* in their tolerance to imidacloprid, although only modest differences in LC_{50} values were observed in the all-stages bioassay (Table 1). Data on tolerances of immature *B. tabaci* to imidacloprid are scarce, but age-specific expression of resistance to neonicotinoid insecticides has been reported to favor adults over immatures.[31] The small size and limited mobility of crawlers and early-instar nymphs may make them more vulnerable to toxic levels of imidacloprid in leaves that adults potentially avoid. Although mortality of adults was generally low on the imidacloprid-treated cantaloupes in the field, sublethal effects such as reduced feeding[32] and oviposition may have occurred. The extent and implications of effects other than acute mortality are poorly known and warrant further study.

5 DISCLAIMER

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