

Assessment of cross-resistance potential to neonicotinoid insecticides in *Bemisia tabaci* (Hemiptera: Aleyrodidae)

N. Prabhaker^{1*}, S. Castle², T.J. Henneberry² and N.C. Toscano¹

¹Department of Entomology, University of California, Riverside, CA 92521, USA; ²Western Cotton Research Laboratory, USDA/ARS, Phoenix, AZ 85040, USA

Abstract

Laboratory bioassays were carried out with four neonicotinoid insecticides on multiple strains of *Bemisia tabaci* (Gennadius) to evaluate resistance and cross-resistance patterns. Three imidacloprid-resistant strains and field populations from three different locations in the southwestern USA were compared in systemic uptake bioassays with acetamiprid, dinotefuran, imidacloprid and thiamethoxam. An imidacloprid-resistant strain (IM-R) with 120-fold resistance originally collected from Imperial Valley, California, did not show cross-resistance to acetamiprid, dinotefuran or thiamethoxam. The Guatemala-resistant strain (GU-R) that was also highly resistant to imidacloprid (RR = 109-fold) showed low levels of cross-resistance when bioassayed with acetamiprid and thiamethoxam. However, dinotefuran was more toxic than either imidacloprid or thiamethoxam to both IM-R and GU-R strains as indicated by low LC₅₀s. By contrast, a Q-biotype Spanish-resistant strain (SQ-R) of *B. tabaci* highly resistant to imidacloprid demonstrated high cross-resistance to the two related neonicotinoids. Field populations from Imperial Valley (California), Maricopa and Yuma (Arizona), showed variable susceptibility to imidacloprid (LC₅₀s ranging from 3.39 to 115 µg ml⁻¹) but did not exhibit cross-resistance to the three neonicotinoids suggesting that all three compounds would be effective in managing whiteflies. Yuma populations were the most susceptible to imidacloprid. Dinotefuran was the most toxic of the four neonicotinoids against field populations. Although differences in binding at the target site and metabolic pathways may influence the variability in cross-resistance patterns among whitefly populations, comparison of whitefly responses from various geographic regions to the four neonicotinoids indicates the importance of ecological and operational factors on development of cross-resistance to the neonicotinoids.

Keywords: whiteflies, imidacloprid resistance, acetamiprid, dinotefuran, thiamethoxam

Introduction

The neonicotinoids are a recently developed class of synthetic insecticides that have been pivotal in protecting crops from some of the world's most serious pests. They are a novel class of insecticides that resemble the natural product nicotine, acting as agonists at the same nicotinic

*Address for correspondence: 4135 E Broadway Road, Phoenix, AZ 85040, USA
Fax: (602) 437 1274
E-mail: nilima.castle@ucr.edu

acetylcholine receptor (nAChR) target site (Tomizawa & Casida, 2003). Neonicotinoids have selective toxicity to insects as foliar or systemic treatments, being especially effective against many hemipteran pests such as aphids, leafhoppers and whiteflies as well as certain chewing insects, notably Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). This new group of chemicals has brought diversity to the insecticide arsenal available for both pest and resistance management and has relieved intensive pressure on older conventional chemicals.

The value of neonicotinoids in pest control continues to increase through discovery of new compounds that may have enhanced activity against pest populations resistant to earlier analogues. While a growing portfolio of products bodes well for growers and pest managers, market positioning of a new neonicotinoid becomes an increasing challenge in an environment of established and proven performers such as imidacloprid. However, the physico-chemical characteristics of the neonicotinoids along with their performance attributes are proving sufficiently variable to allow ample pest management opportunities. The key to successful marketing in the crop protection environment may be a thorough understanding of the activity range of related compounds in various cropping environments focusing on their strengths and weaknesses.

As part of the larger process of determining the potential of related neonicotinoids against particular pests, the present study was initiated to explore their performance against *B. tabaci* (Gennadius) (Hemiptera: Aleyrodidae), one of the most serious agricultural pests worldwide. In the American southwest, *B. tabaci* has been the principal pest of vegetable and field crops for many years. Devastating outbreaks in the early 1990s in California, Texas and Arizona resulted in lost farm revenues in excess of hundreds of millions of dollars (Castle *et al.*, 1995). The first commercial use of imidacloprid in 1993 in California soon became the foundation of a chemical management programme that has strengthened over time with registration of new compounds including two insect growth regulators and at least two additional neonicotinoids (Castle *et al.*, 2002). The resurgence of conventional chemistry has also helped to keep *B. tabaci* populations in check. Although concerns about resistance to imidacloprid and other compounds arise from time to time, the outlook for continued successful management of *B. tabaci* in the southwest is overall good.

However, concerns over potential resistance development to compounds in this group have been expressed, especially in light of the heavy reliance that has been placed upon imidacloprid. A well-documented case of resistance to imidacloprid has been reported from a region facing chronic whitefly problems in Almeria, Spain, a place of intensive year-round vegetable production on more than 30,000 ha of protected agriculture (Elbert & Nauen, 2000). A prevalence of plant viral diseases in tomatoes and cucumber has resulted in reduced action thresholds and intensified chemical treatments that have exacerbated the resistance problem. Cucurbit viral diseases have had a similar impact on the intensity of insecticide use in Guatemala. In recent years, continuous melon production between September and May has been hampered by large infestations of *B. tabaci* and the viruses they transmit. The multiple uses of imidacloprid throughout the melon cropping cycles coupled with long persistence in treated plants have placed this particular

compound and perhaps subsequent neonicotinoids at a high risk for resistance development.

At present, acetamiprid, imidacloprid and thiamethoxam are being used extensively because of their effectiveness against important pests like whiteflies and aphids. Because of possible shared target sites and similar degradation pathways among neonicotinoid compounds, the major concern with intensive use of these products is the potential for cross-resistance. Development of resistance to one neonicotinoid compound may constitute a threat to all members of this class. Efforts should be directed toward identifying cross-resistance patterns to specific compounds in this group. Expanding use of neonicotinoid products against agricultural pests on cross-commodity crops will increase the risk for resistance. Thus, there is an urgent need for integrating the neonicotinoids into a diversified programme of chemical control to avoid high selection pressure on any one chemical and indeed on the whole class of neonicotinoids. Basic studies to identify cross-resistance patterns within this class can contribute to a set of guidelines that will help to prevent or forestall resistance.

Towards this goal, evaluations were conducted on three different imidacloprid-resistant strains of *B. tabaci* in a series of bioassays with acetamiprid, dinotefuran, imidacloprid and thiamethoxam to examine the degree of cross-resistance between them. Additionally, a number of *B. tabaci* populations collected from imidacloprid-treated fields were tested simultaneously against all four neonicotinoids to evaluate relative toxicities as well as determine if any cross-resistance patterns were apparent among the different populations tested.

Materials and methods

Resistant whitefly strains

Whitefly strains tested from Arizona, California and Guatemala were the B-biotype while the SQ-R strain from Spain was a Q-biotype.

Imidacloprid-resistant strain (IM-R)

An imidacloprid-resistant strain of *B. tabaci* was developed by selectively breeding for imidacloprid resistance (Prabhaker *et al.*, 1997). Initially, whitefly pupae on melon leaves were collected in May 1993 from an imidacloprid-treated melon field at the USDA Research Station in Imperial Valley, California, and used to initiate this strain by treating the soil systemically with imidacloprid. It has been maintained in the laboratory under selection to imidacloprid for approximately 9 years. At the time of this study the resistance level to imidacloprid in this strain was about 120-fold.

Guatemala-resistant strain (GU-R)

Melon crops are grown in Guatemala sequentially beginning in September until the final planting is harvested in May. After receiving reports of poor whitefly control on imidacloprid-treated melons, whitefly samples were shipped to our laboratory for evaluation. Melon leaves infested with whitefly pupae were collected from imidacloprid-treated melon fields and received from Guatemala at various times in 2000/2001. The whitefly-infested leaves were held in large cages (152 × 101 × 116 cm) for 3–5 days to allow emergence of

adults. Fresh, uninfested melon plants were placed in the cage to host newly emerged adults. Bioassay tests were conducted on newly emerged adults from the field collections and on the first generation (F_1) adults following establishment in the greenhouse. This strain has been maintained under periodic selection with systemic applications of imidacloprid. Comparative toxicity tests between the four neonicotinoids were conducted to assess cross-resistance patterns in the adults of this colony.

Spanish-resistant strain (SQ-R)

Collections of the Q type adult whiteflies were made on poinsettia in Almeria, Spain, in 2003 and transported back in containers with fresh leaves to the quarantine laboratory in Riverside, California, for establishment on cotton. It is well documented that whiteflies from Almeria, Spain, were highly resistant to imidacloprid and most of the conventional insecticides (Elbert & Nauen, 2000) that have been used routinely in the area's vegetable crops. The presence of the Q biotype in Spain was first demonstrated with the use of esterase patterns which differed from those of the B biotype (Guirao *et al.*, 1997; Banks *et al.*, 1998).

Field collections of whiteflies

A number of adult populations of whiteflies were collected over a period of 10–12 months from selected regions of Arizona and Imperial Valley, California, USA. Collections of adults were made from broccoli at the Maricopa Agricultural Center, Arizona (MAC), from melons in Yuma, Arizona (YUM) and from cotton and melons in Imperial Valley, California (IV), for determining the cross-resistance data to four neonicotinoids. Adult whiteflies were vacuumed from the crops and transported back to the laboratory on cotton plants confined within a transfer cage. All test subjects were used in the bioassay the day after collection.

Reference strain

The reference strain of *B. tabaci* was collected in Imperial Valley on untreated cotton in 1998. This strain has been in laboratory culture on cotton without any exposure to insecticides for > 40 generations when this study was initiated. The reference strain was used to compare the resistance levels to the four neonicotinoids.

Systemic uptake bioassays

A standard procedure was developed that used excised cotton leaves to determine the systemic action of imidacloprid and the cross-resistance patterns to four neonicotinoids in whiteflies. This simple uptake bioassay enables exposure of test insects to the systemic activity of these compounds. To minimize variation due to size or age, cotton leaves from the first or second node of a 5–6 true leaf cotton plant were used in this technique for assessment of toxicity. Appropriate concentrations of each insecticide were prepared on the day of treatment and 9.5 ml aliquots of each dilution was placed in an aquapik. The excised leaves of cotton were placed in serial dilutions of each test compound in aquapiks for 24 h. This technique allows uptake of each compound through the petioles directly avoiding any problems related to binding in soil for translocation of the compounds in the leaves. After

24 h uptake of each insecticide, treated leaves were transferred to a duplicate set of aquapiks containing water only. The control leaves were placed in water alone. For exposure to the four compounds, 30–40 unsexed whitefly adults were aspirated into small clip cages (7 cm diameter) that were attached to the treated leaves. At least four replicates at each concentration for each test chemical were established and a minimum of six concentrations plus untreated controls were included in each test. Mortality counts were made after 24 and 48 h. The criterion for mortality was the failure of an adult to fly when probed. All tests were conducted and maintained at $26 \pm 1^\circ\text{C}$ under 12:12 (L:D) cycle.

Insecticides

The following four neonicotinoid insecticides of formulated grade were provided by the respective manufacturing company: (i) acetamiprid (Intruder® 70% AI) from DuPont, Wilmington, Delaware, USA (ii) dinotefuran (Venom® 2EC) from Valent, Walnut Creek, California, USA (iii) imidacloprid (Admire® 2F) from Bayer Ag, Kansas City, Missouri, USA and (iv) thiamethoxam (Platinum® 25C) from Syngenta (formerly Novartis), Oxnard, California. Stock and serial dilutions for the formulated compounds were made with water on the day of tests for use in systemic bioassays. Technical grade acetamiprid was also obtained from DuPont, Wilmington, Delaware, for tests against the IM-R strain alone. A 1% ($10,000 \mu\text{g ml}^{-1}$) stock solution of technical acetamiprid was made in acetone but serial dilutions were made in water alone.

Statistical analysis

Results of the dose-mortality experiments were analysed using the POLO programme (Russell *et al.*, 1977) to obtain LC_{50} and LC_{90} values. Differences in LC_{50} and LC_{90} values were considered to be significant if there was no overlap in the confidence limits. Resistance ratios were calculated by dividing the respective LC_{50} of each resistant strain for each compound by the LC_{50} of a reference strain.

Results

Cross-resistance in the IM-R strain to neonicotinoids

Bioassays of the IM-R strain with the four neonicotinoid compounds produced a wide range of responses as measured by LC_{50} s at 24 and 48 h exposure (table 1). For imidacloprid, an LC_{50} of $498 \mu\text{g ml}^{-1}$ at 24 h post-treatment period indicated a high level of resistance ($\text{RR} = 160$) (table 1). At the 48 h reading, higher mortality was recorded ($\text{LC}_{50} = 293 \mu\text{g ml}^{-1}$, $\text{RR} = 120$), but no additional mortality was observed after 72 h. In the acetamiprid bioassay, higher mortality compared to imidacloprid ($\text{LC}_{50} = 139 \mu\text{g ml}^{-1}$) was observed after 24 h. Additionally, with longer exposure, mortality at 48 h increased considerably as indicated by a lower LC_{50} of $11 \mu\text{g ml}^{-1}$. Similar to the activity of acetamiprid, thiamethoxam was slower in action against IM-R whiteflies at 24 h, but mortality increased by 48 h to yield an LC_{50} of $10 \mu\text{g ml}^{-1}$ ($\text{RR} = 2$, non-significant based on overlap of 95% CI). In contrast to the other three compounds, IM-R whiteflies were much more susceptible to dinotefuran at 24 h with an LC_{50} of $3.72 \mu\text{g ml}^{-1}$. However, even with the

Table 1. Toxicity of four neonicotinoids to *Bemisia tabaci* adults of the IM-R strain.

Chemical	Time (h)	Sample no.	Slope \pm SE	LC 50 $\mu\text{g ml}^{-1}$ (95% CI)	LC 90 $\mu\text{g ml}^{-1}$ (95% CI)	^a RR at LC ₅₀
Imidacloprid	24	972	3.1 \pm 0.26	498 (281–839)	1102 (454–1526)	160
	48		3.7 \pm 0.03	293 (198–381)	843 (454–1126)	120
Acetamiprid	24	1060	1.6 \pm 0.20	139 (85–176)	817 (590–1311)	8
	48		1.8 \pm 0.38	11 (6.4–36.2)	78 (59–164)	5
Dinotefuran	24	1225	2.9 \pm 0.28	3.72 (1.26–12.78)	99 (58–632)	1
	48		3.4 \pm 0.14	0.098 (0.008–0.043)	4.56 (1.07–9.63)	1
Thiamethoxam	24	1064	2.7 \pm 0.12	239 (137–542)	994 (691–1238)	22
	48		2.3 \pm 0.46	10 (4.91–58.5)	103 (73–189)	2

^aRR = resistance ratio obtained by the LC₅₀ of each compound against IM-R divided by the LC₅₀ of a reference strain at 14.29 $\mu\text{g ml}^{-1}$ (24 h) and 2.02 $\mu\text{g ml}^{-1}$ (48 h) for acetamiprid, 2.86 $\mu\text{g ml}^{-1}$ (24 h) and 0.098 $\mu\text{g ml}^{-1}$ (48 h) for dinotefuran, 3.11 $\mu\text{g ml}^{-1}$ (24 h) and 2.43 $\mu\text{g ml}^{-1}$ (48 h) for imidacloprid and 10.64 $\mu\text{g ml}^{-1}$ (24 h) and 4.54 $\mu\text{g ml}^{-1}$ (48 h) for thiamethoxam.

low LC₅₀ at 24 h, dinotefuran was significantly more toxic after 48 h to the IMR strain (LC₅₀ = 0.098 $\mu\text{g ml}^{-1}$). In general, toxicity to each neonicotinoid increased after 48 h exposure in the reference strain (table 1).

Cross-resistance to neonicotinoids in the GU-R strain

Toxicity tests showed that GU-R adults were resistant to imidacloprid based on a high LC₅₀ of 264 $\mu\text{g ml}^{-1}$ (RR = 109) at 48 h exposure (table 2). Toxicity did not increase significantly after 48 h. In contrast to toxicity of imidacloprid, dinotefuran proved to be highly effective against the GU-R strain (LC₅₀ = 4.7 $\mu\text{g ml}^{-1}$), demonstrating little cross-resistance between imidacloprid and dinotefuran (RR = 2). Toxicity increased slightly (LC₅₀ = 1.79 $\mu\text{g ml}^{-1}$) after 48 h to dinotefuran. Compared to the LC₅₀ values of dinotefuran of

the GU-R strain, acetamiprid was approximately ten times less toxic against this strain as indicated by a significantly higher LC₅₀ value of 45.6 $\mu\text{g ml}^{-1}$ at 48 h post-treatment (RR = 23) (table 2). Similarly, thiamethoxam was also significantly less effective by 21-fold (LC₅₀ = 108 $\mu\text{g ml}^{-1}$). Resistance ratios obtained for acetamiprid and thiamethoxam based on LC₅₀s at 24 h are not considered as true resistance because mortality increased significantly after 24 h. Results suggest variable levels of cross-resistance in the GU-R strain between the four neonicotinoids (RR ranging from 23 to 24-fold).

Cross-resistance to neonicotinoids in the SQ-R strain

In contrast to the responses of the IM-R and GU-R strains, experimental tests of the four neonicotinoids against the

Table 2. Toxicity of four neonicotinoids against *Bemisia tabaci* adults of the GU-R strain.

Chemical	Time (h)	Sample no.	Slope \pm SE	LC 50 $\mu\text{g ml}^{-1}$ (95% CI)	LC 90 $\mu\text{g ml}^{-1}$ (95% CI)	^a RR at LC ₅₀
Imidacloprid	24	938	3.1 \pm 0.21	302 (192–391)	644 (496–882)	–
	48		3.2 \pm 0.23	264 (202–373)	652 (403–874)	109
Acetamiprid	24	887	2.1 \pm 0.41	292 (166–712)	576 (398–885)	–
	48		2.3 \pm 0.20	45.6 (19–93)	233 (132–412)	23
Dinotefuran	24	990	3.1 \pm 0.32	4.71 (1.02–10.97)	22.08 (5.90–64.5)	2
	48		2.9 \pm 0.28	1.79 (0.91–4.65)	20.14 (7.92–37.56)	1
Thiamethoxam	24	898	2.3 \pm 0.18	153 (97–242)	461 (256–578)	–
	48		2.5 \pm 0.25	108 (48.5–245)	403 (273–786)	24

^aRR = resistance ratio obtained by the LC₅₀ of each compound against GU-R divided by the LC₅₀ of a reference strain at 14.29 $\mu\text{g ml}^{-1}$ (24 h) and 2.02 $\mu\text{g ml}^{-1}$ (48 h) for acetamiprid, 2.86 $\mu\text{g ml}^{-1}$ (24 h) and 0.098 $\mu\text{g ml}^{-1}$ (48 h) for dinotefuran, 3.11 $\mu\text{g ml}^{-1}$ (24 h) and 2.43 $\mu\text{g ml}^{-1}$ (48 h) for imidacloprid and 10.64 $\mu\text{g ml}^{-1}$ (24 h) and 4.54 $\mu\text{g ml}^{-1}$ (48 h) for thiamethoxam.

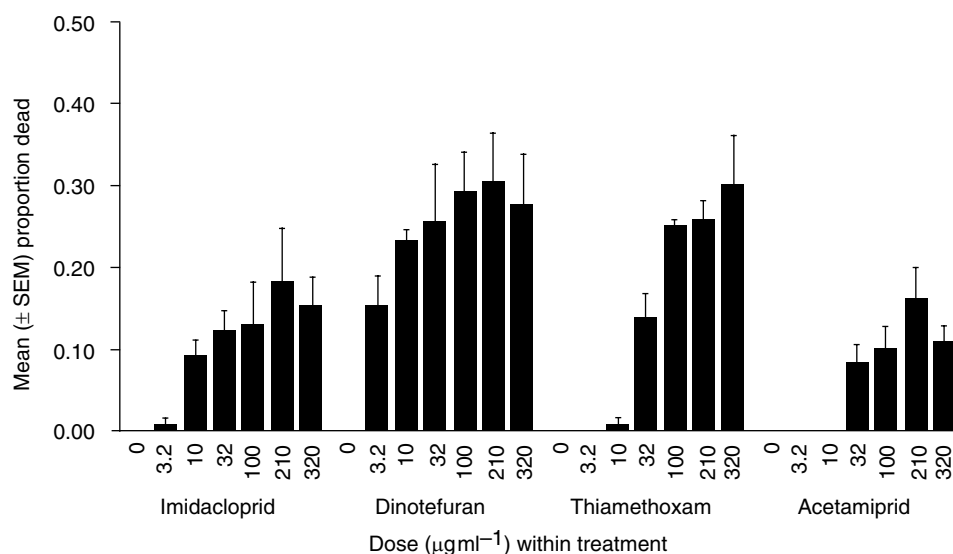


Fig. 1. Mean (\pm SEM) mortality responses of the Q biotype of *Bemisia tabaci* from Spain to four neonicotinoid insecticides.

SQ-R strain produced evidence of strong cross-resistance. Resistance was quite high and there were little dose-mortality responses to any of the four compounds, leading to rejection of the probit model when applied to the toxicity data for three of the four compounds (Robertson & Priesler, 1992). Adults of the SQ-R strain were highly resistant to imidacloprid as indicated by an extremely low mean mortality of 16% even at the highest dose of $320 \mu\text{g ml}^{-1}$ (fig. 1). The highest mean mortality observed with imidacloprid was only 19% at $210 \mu\text{g ml}^{-1}$. Adults of the SQ-R strain were slightly more susceptible to dinotefuran at the same concentration compared with the toxicity of imidacloprid as shown by a mean mortality of 30%, but the difference was not significant. Similarly, SQ-R adults showed equal susceptibility of 30% mean mortality to thiamethoxam but at a higher concentration of $320 \mu\text{g ml}^{-1}$ without significance. Among the four neonicotinoids, acetamiprid was the least effective compound against the adults of this strain. Mortality was extremely low (11%) at the high dose of $320 \mu\text{g ml}^{-1}$ suggesting that these insects were highly cross-resistant to this compound. Bioassay results suggest the presence of strong cross-resistance among the four neonicotinoids in SQ-R adults in contrast to cross-resistance patterns of IM-R adults.

Cross-resistance patterns to neonicotinoids in field populations of *B. tabaci*

Arizona field populations (MAC and YUM)

Adult whiteflies collected from Maricopa Agricultural Center (MAC) and Yuma (YUM) in Arizona varied considerably in susceptibility to three neonicotinoids. However, dinotefuran proved to be more toxic than either imidacloprid or thiamethoxam in most comparisons based on statistically lower LC_{50} s. Initial tests of *B. tabaci* adults collected in November 2003 on broccoli at MAC (Central Arizona) yielded a mean mortality of 90% to dinotefuran and 85% to thiamethoxam at the $100 \mu\text{g ml}^{-1}$ concentration but only 50% to imidacloprid. The LC_{50} for imidacloprid in this

test was $89 \mu\text{g ml}^{-1}$, >8-fold higher than the LC_{50} of $10.5 \mu\text{g ml}^{-1}$ for dinotefuran and nearly 5-fold higher than the LC_{50} of $18.9 \mu\text{g ml}^{-1}$ for thiamethoxam (fig. 2a). After establishing a greenhouse colony from this autumn collection, whiteflies were again bioassayed 4 months later in April

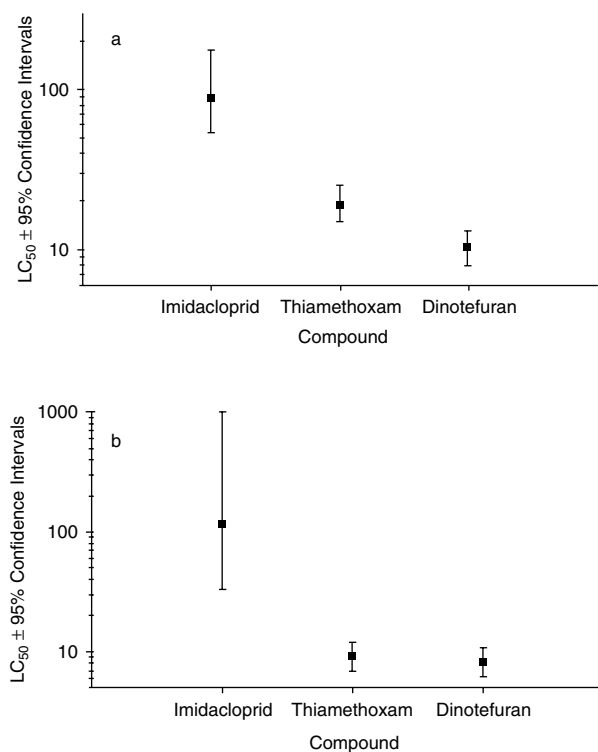


Fig. 2. Dose-mortality responses expressed as LC_{50} ($\mu\text{g ml}^{-1}$) of *Bemisia tabaci* from the Maricopa Agricultural Center, Arizona, USA to three neonicotinoid insecticides in November 2003 (a) and April 2004 (b).

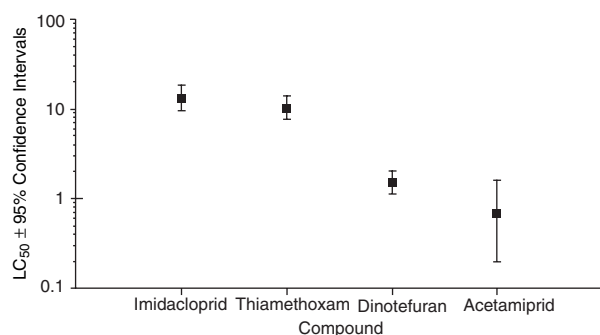


Fig. 3. Dose-mortality responses expressed as LC₅₀ (µg ml⁻¹) of *Bemisia tabaci* from Yuma, Arizona, USA to four neonicotinoid insecticides in July 2004.

2004 to yield relative toxicities similar to those obtained in the previous test (fig. 2b). In contrast to the higher mortalities observed at the highest concentrations for dinotefuran and thiamethoxam, mortality levelled off for imidacloprid to yield an LC₅₀ of 115 µg ml⁻¹, 14- and 12-fold greater than LC₅₀s for dinotefuran (8.2 µg ml⁻¹) and thiamethoxam (LC₅₀ = 9.4 µg ml⁻¹), respectively.

The first collection of YUM whiteflies (dispersing adults from area alfalfa fields that were concentrated on home ornamentals) in early October 2003 proved to be so susceptible to imidacloprid that over 95% of all insects were dead at the 1 µg ml⁻¹ concentration but with <5% control mortality (data not shown). Because of the high susceptibility of the first field-collected whiteflies, the dosage range for the following test was lowered to obtain a better dose/mortality response. Whiteflies collected from a melon field in Yuma in November 2003 also proved to be highly susceptible to both imidacloprid and dinotefuran with low LC₅₀ values of 2.6 and 0.51 µg ml⁻¹ respectively. Simultaneous bioassays conducted with all four compounds on *B. tabaci* collected in July 2004 (fig. 3) following their development on imidacloprid-treated spring cantaloupes yielded significantly higher LC₅₀s for both imidacloprid (13.1 mg ml⁻¹) and thiamethoxam (10.2 µg ml⁻¹) than for dinotefuran (1.5 µg ml⁻¹) or acetamiprid (0.7 µg ml⁻¹).

Imperial Valley whiteflies

Adult whitefly populations from Imperial Valley were sampled and bioassayed both in spring and summer of 2004 to evaluate relative toxicities of four neonicotinoids. In the early season sample collected from spring cantaloupes in April, thiamethoxam (LC₅₀ = 6.9 µg ml⁻¹) was significantly less toxic to adult *B. tabaci* than any of the other three compounds (fig. 4a). There was a slight overlap of 95% CI between imidacloprid (LC₅₀ = 13.9 µg ml⁻¹) and dinotefuran (LC₅₀ = 3.4 µg ml⁻¹) but acetamiprid (LC₅₀ = 2.1 µg ml⁻¹) proved significantly more toxic than all others. In contrast to the early season populations, adult whiteflies collected in late June on imidacloprid-treated cantaloupes were significantly more tolerant to imidacloprid (LC₅₀ = 31.3 µg ml⁻¹) than to the other three compounds (fig. 4b). Thiamethoxam (LC₅₀ = 5.27 µg ml⁻¹) was intermediate in toxicity while dinotefuran (LC₅₀ = 0.7 µg ml⁻¹) and acetamiprid (LC₅₀ = 0.6 µg ml⁻¹) again demonstrated high toxicity on the order of 45- and 52-fold greater than imidacloprid.

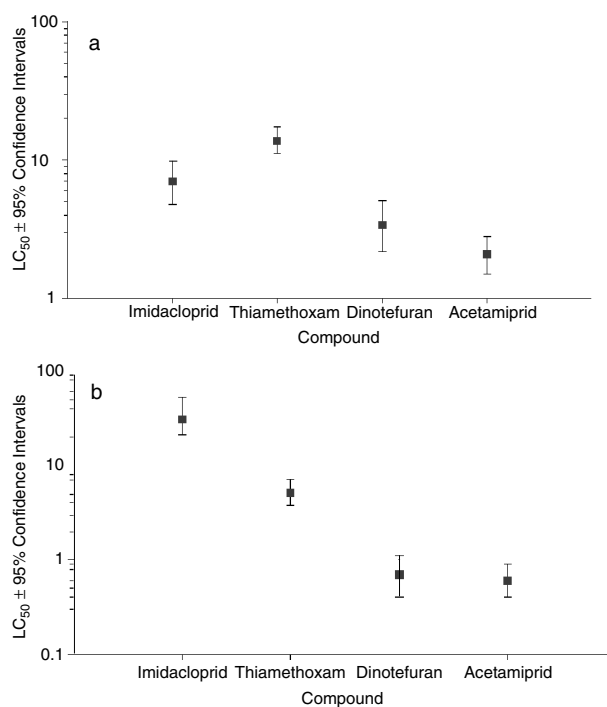


Fig. 4. Dose-mortality responses expressed as LC₅₀ (µg ml⁻¹) of *Bemisia tabaci* from Imperial Valley, California, USA to four neonicotinoid insecticides in April 2004 (a) and July 2004 (b).

Discussion

Cross-resistance patterns varied among the four neonicotinoid insecticides when tested on both natural populations and imidacloprid-resistant strains of *B. tabaci* in systemic uptake bioassays. All three resistant strains showed high resistance to imidacloprid, but by far the highest resistance was observed in the SQ-R strain. Bioassay results on the SQ-R in three different tests yielded dose-mortality responses that were too flat to satisfy the assumptions of the probit model for all compounds except for imidacloprid in the first test. In this case, mortality was quite low at 16% at the high dose of 320 µg ml⁻¹ for imidacloprid in the Q-biotype of *B. tabaci* from Almeria. These results confirm previous reports of Q-type whiteflies from Almeria being highly resistant to imidacloprid with little regression towards susceptibility (Cahill *et al.*, 1996; Elbert & Nauen, 2000; Nauen *et al.*, 2002). Moreover, they also demonstrate high cross-resistance to other three neonicotinoids. Mortality of the SQ-R strain to dinotefuran was at 30% at the high concentration of 320 µg ml⁻¹, similar to the mortality range for imidacloprid. Similarly, although no resistance ratios were calculated for thiamethoxam and acetamiprid in this strain, cross-resistance was high between the two compounds. Our results validate previous reports of cross-resistance to neonicotinoids in Q-type whitefly populations from Spain, Italy and Germany (Nauen *et al.*, 2002). In their study a 100-fold cross-resistance to thiamethoxam and acetamiprid in Almeria whiteflies was determined, while cross-resistance to neonicotinoids was stable in the Q-type strains from Germany and Italy. In the same study, it was reported that the mechanism of resistance to neonicotinoids

in Q-type *B. tabaci* was not associated with a lower affinity of imidacloprid to nicotinic acetylcholine receptors. It was suggested that it was probably due to oxidative degradation by cytochrome P450-dependent monooxygenases based on synergistic studies with piperonyl butoxide and phenylphosphonate (Nauen *et al.*, 2002; Stumpf & Nauen, 2002). Due to the nature of the cytochrome P450-dependent monooxygenases complex that has broad substrate specificity and depending on the levels of this enzyme complex in Q-type whiteflies, it is possible that this resistance to imidacloprid extends to other related neonicotinoids, as observed in our study. Similarly, detoxification due to increased levels of monooxygenases was considered to be an important mechanism of resistance to imidacloprid in *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) (Zewen *et al.*, 2003). It is also known that imidacloprid and other neonicotinoids undergo oxidative detoxification in plants and vertebrates resulting in inactive metabolites (Araki *et al.*, 1994; Nauen *et al.*, 1999). Although piperonyl butoxide did not suppress cross-resistance to imidacloprid in a multi-resistant strain of houseflies highly resistant to pyrethroids, it did in an abamectin-resistant strain of houseflies, suggesting that monooxygenases were the mechanism responsible for cross-resistance to imidacloprid (Wen & Scott, 1997). In contrast, a mechanism of target site insensitivity has been suggested in a strain of *L. decemlineata* showing 150-fold resistance to imidacloprid and a low cross-resistance ratio of 3-fold to thiamethoxam (Hollingworth *et al.*, 2002). In general, resistance to neonicotinoids appears to be due to enhanced oxidative detoxification.

Based on the LC₅₀s, resistance levels to imidacloprid in IM-R and GU-R strains of *B. tabaci* were determined to be 120- and 109-fold, respectively. Despite the presence of high resistance to imidacloprid in the two strains, cross-resistance to dinotefuran was low. Dinotefuran, structurally distinct from imidacloprid, has been reported to have high insecticidal activity against a broad range of hemipteran insects (Kiryama & Nishimura, 2002). Cross-resistance to both acetamiprid and thiamethoxam was also low and statistically non-significant in the IM-R strain. In contrast to the IM-R strain, moderate levels of cross-resistance to acetamiprid (RR=23) and thiamethoxam (RR=24) were present in the GU-R strain. A common resistance mechanism to one compound can confer cross-resistance to other compounds (Georghiou, 1965). Imidacloprid had been applied at various intervals throughout the melon-growing seasons in Guatemala subjecting the whiteflies to heavy selection pressure for a few years. These results are not surprising considering the heavy usage of imidacloprid on one cropping system for 9 months of the year. Also, resistance to imidacloprid was stable for many months in the GU-R strain while under maintenance in the laboratory without regular selection by imidacloprid. Both factors may have influenced the development of some cross-resistance to various insecticides that target nicotinic acetylcholine receptor sites. Similar results of cross-resistance extending to related neonicotinoids, imidacloprid analogues, acetamiprid and monosultap were observed in *N. lugens* (Zewen *et al.*, 2003). Strategies to combat neonicotinoid resistance must take account of the cross-resistance characteristics of these mechanisms, the ecology of target pests on different host plants, and the implications of increasing diversification of the neonicotinoid new molecules.

Monitoring data of field populations from Arizona and California showed variable LC₅₀ values to imidacloprid depending on the season during which collections were made. Higher LC₅₀ values to imidacloprid were observed in summer collections compared with autumn groups based on dose-mortality regressions. These results suggest that susceptibility to imidacloprid was unstable in these populations and may be linked to crop sequences that change during the year in Imperial Valley and Yuma. Therefore, the lack of extension of cross-resistance between the four neonicotinoids in field populations of whiteflies from both Arizona and California with the exception of MAC insects, was not surprising. Despite the magnitude of imidacloprid use as soil applications to control whiteflies on multiple crops in Imperial Valley and Yuma, high resistance to imidacloprid has not been recorded to date. Generally, selection pressure by systemic insecticides towards resistance build-up is much higher than foliar sprays because of longer residual activity, exposure of all stages of a pest (Taylor & Georghiou, 1982) as well as survival at sublethal dosages leading to selection for resistance over time. This theory was validated by artificially selecting whiteflies for high resistance to imidacloprid with systemic treatment for a number of generations under laboratory conditions (Prabhaker *et al.*, 1997). The above assumption was not true of field populations of *B. tabaci*. Previously, spatial and temporal monitoring of *B. tabaci* populations within the cropping systems in Imperial Valley and Yuma has shown that there was no resistance to imidacloprid or other conventional chemicals in spite of heavy use for a number of years (Castle *et al.*, 1995, 2002; Prabhaker *et al.*, 1997). On the contrary, susceptibility to all commonly used insecticides including imidacloprid increased with time in the Imperial Valley populations. Similarly, in Israel, no cross-resistance between imidacloprid and acetamiprid was observed in whiteflies even after two years of use in cotton (Horowitz *et al.*, 1998). These results indicate the importance of factors other than commonality of chemical structure-relationships or the mode of action within a class of insecticide chemistry. Biological, operational, environmental and/or ecological factors could influence the development of resistance in a pest (Georghiou & Taylor, 1977a,b) and as such the differences in ecological factors among the various regions from which whiteflies originated may explain some of the observed variation in patterns of cross-resistance among neonicotinoids.

In this study, whiteflies evaluated for cross-resistance patterns to neonicotinoids originated from varied habitats. Comparison of ecological factors and management practices in each region suggest their influence on the dynamics of resistance development to neonicotinoids and also how heritable features of individual populations are important in differentiating between populations from various geographic locations. In general, pest systems are highly variable in time and space and are affected by dispersal. For example, the various field populations from Imperial Valley and the IM-R strain originated from a region where the cropping patterns and operational practices encouraged the mitigation of resistance development to many insecticides, including neonicotinoids (Castle *et al.*, 2002). For example, large areas averaging 200,000 acres of alfalfa that are left untreated are grown in Imperial Valley compared with a small area of approximately 5000 acres of field crops that are treated. This generates a large reservoir of susceptible

genotypes because whiteflies do reproduce on alfalfa during summer months. Additionally, whiteflies have a propensity for high migration rates moving from treated to untreated crops and vice versa. This can result in the dilution of resistance genes and may block or delay resistance to some extent. A delay in resistance could also be related to the cropping patterns maintained in Imperial Valley where melons are grown in spring, cotton in summer and cole crops in autumn. As a result, insecticides are alternated during each season, thus avoiding heavy selection pressure by any one insecticide or class of insecticide. A combination of these factors may be delaying resistance to neonicotinoids in whiteflies in spite of the magnitude of imidacloprid use in Imperial Valley. Similar conditions of cropping systems and management practices also exist in the Yuma region, hence the absence of neonicotinoid resistance to date and lack of cross-resistance between the neonicotinoids in YUM whiteflies. Differences in cropping systems exist between the Maricopa and Yuma regions of Arizona. Unlike the cropping patterns in Yuma, the more prominent crop grown in Maricopa is cotton. Cultural practices and ecology in the Guatemala region are contrary to that of Imperial and Yuma regions. A monoculture of melon crops is maintained for 9 months of the year. This translates into continual applications of insecticides, especially imidacloprid, throughout the 9-month period. Since there are no alternate untreated host plants to allow maintenance of susceptible genotypes, coupled with heavy selection pressure under imidacloprid treatments, resistance to imidacloprid evolved, which in turn extended in part to related neonicotinoids as was observed in the present study. Some similarity is noted in comparing the cropping systems and management practices of the Almeria region of Spain and Guatemala. In Almeria, high value vegetable crops are grown on 30,000 ha, synchronously throughout the year in open plastic houses which result in a closed system of selecting whiteflies with heavy imidacloprid (Confidor) treatments that are being applied to 60% of the area to manage whiteflies (Cahill & Denholm, 1999). As such, results showing high resistance levels to neonicotinoids in whiteflies from this region observed in this and other studies are not surprising and there seems to be no chance of reducing the total insecticide pressure due to continuous vegetable production. High resistance to neonicotinoids in the SQ-R strain could also be partly due to lack of refugia from untreated host plants that might dilute resistance genotypes and thus delay resistance and cross-resistance to neonicotinoids as observed in Imperial Valley and Yuma. In addition to the aforementioned reasons, high and stable resistance due to target site insensitivity to older chemicals has been reported (Denholm *et al.*, 1996) which may have contributed towards increase in neonicotinoid resistance in this biotype of *B. tabaci*.

In summary, the levels of cross-resistance among neonicotinoids recorded in the present study in *B. tabaci* showed no consistent patterns. Minor differences were observed in some cases and even significant differences were recorded in the Spanish Q-type *B. tabaci*, indicating that each insecticide and resistance may have very different properties under different ecological environments. Therefore, assessing risk for cross-resistance potential based on comparisons between agroecosystems and natural systems are difficult to make because factors such as weather, host plants, plant genetics and the role of natural enemies vary. However, if the ecological systems and practices have been fairly stable, such

as in the case of the Imperial Valley or Yuma regions, it may be possible to make knowledgeable recommendations based on extrapolations from such studies to combat neonicotinoid resistance. Knowledge of patterns of cross-resistance within the neonicotinoid class are important to determine whether members of this class might be alternated without resulting in continuous selection for the same resistance mechanism.

Acknowledgements

Partial funding was provided for this work by Syngenta (formerly Novartis, Basel, Switzerland).

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(Accepted 21 July 2005)

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