Research Article

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Toxicity of spiromesifen to the developmental stages of *Bemisia tabaci* biotype B

Svetlana Kontsedalov,[a] Yuval Gottlieb,[a] Isaac Ishaaya,[a] Ralf Nauen,[b] Rami Horowitz[c] and Murad Ghanim[a]*

Abstract

BACKGROUND: Spiromesifen is a novel insecticidal/acaricidal compound derived from spirocyclic tetronic acids that acts effectively against whiteflies and mites via inhibition of acetyl-CoA-carboxylase, a lipid metabolism enzyme. The effects of spiromesifen on the developmental stages of the whitefly *Bemisia tabaci* (Gennadius) were studied under laboratory conditions to generate baseline action thresholds for field evaluations of the compound.

RESULTS: Adult *B. tabaci* mortality rate after spiromesifen treatment (5 mg L^{-1}) was 40%. Treatment with 0.5 mg L^{-1} reduced fecundity per female by more than 80%, and fertility was almost nil. LC_{50} for eggs was 2.6 mg L^{-1}, and for first instar 0.5 mg L^{-1}. Scanning electron microscopy revealed that eggs laid by treated adult females had an abnormally perforated chorion, and females were unable to complete oviposition. Light and fluorescent microscopy showed significantly smaller eggs following treatment, and smaller, abnormally formed and improperly localized bacteriomes in eggs and nymphs. The number of ovarioles counted in females treated with 5 mg L^{-1} was significantly reduced. Spiromesifen showed no cross-resistance with other commonly used insecticides from different chemical groups, and resistance monitoring in Israel showed no development of field resistance to this insecticide after 1 year of use.

CONCLUSION: The strong effect on juvenile stages of *B. tabaci* with a unique mode of action and the absence of cross-resistance with major commonly used insecticides from different chemical groups suggest the use of spiromesifen in pest and resistance management programmes.

Keywords: *Bemisia tabaci*; spiromesifen; insecticide; resistance

1 INTRODUCTION

The sweet potato whitefly *Bemisia tabaci* (Gennadius) and its biotypes have become a major threat to agriculture worldwide, particularly ornamental, vegetable and field crops, because of the direct damage that they inflict and their ability to transmit major disastrous viruses.[1,2] Although broad-spectrum insecticides such as organophosphates, carbamates and pyrethroids have been used to control the whitefly,[3–5] their toxicity to humans and beneficial organisms and the development of resistance among *B. tabaci* biotypes have led companies to develop 'environmentally friendly' pesticides. These new insecticides specifically target insect pests and have become major components in pest and resistance management programmes.[6–8] The extensive use of new insecticides against *B. tabaci*, such as first (imidacloprid) and second (acetamiprid and thiamethoxam) classes of neonicotinoids,[9–11] and insect growth regulators (benzoylphenyl ureas, pyriproxyfen) in cropping systems is approaching a point at which resistance problems and field failures are just a matter of time.[12–14] Several studies have already reported resistance problems with some of these compounds, and thus new chemical molecules that specifically target insect pests and exhibit low toxicity to the environment are needed. One of the new classes that have recently been developed is tetronic acid derivatives. Spiromesifen is an insecticide from the new class of spirocyclic tetronic acids that acts effectively against whiteflies and mites.[14–17] It acts as an inhibitor of acetyl-CoA-carboxylase, a lipid metabolism enzyme,[16] and causes a significant decrease in total lipids. This compound has been introduced in several countries over the last few years and is becoming an important compound for controlling whiteflies and mites in resistance management programmes, along with other effective insecticides such as neonicotinoids and diafenthiuron. Several recent studies have shown the effectiveness of spiromesifen against whiteflies and mites.[16,17] In the present study, the effects of spiromesifen were evaluated under laboratory and field conditions on different developmental stages of *B. tabaci*, as well as on their fertility and fecundity. Contact, translaminar and systemic activities were also tested. The results suggest that spiromesifen is a much more active compound against *B. tabaci* ova than was

* *Correspondence to: Murad Ghanim, Institute of Plant Protection, Department of Entomology, The Volcani Centre, Bet Dagan 50250, Israel. E-mail: ghanim@agri.gov.il*

a *Department of Entomology, Agricultural Research Organization, Bet Dagan 50250, Israel*

b *Bayer CropScience AG, Research, Biology Insecticides, Alfred Nobel Str. 50, D-40789, Monheim, Germany*

c *Department of Entomology, Agricultural Research Organization, Gilat Research Centre, MP Negev, 85280, Israel*

previously thought, and as such it can be considered to be an effective compound for use in resistance management programmes against *B. tabaci*.

2 EXPERIMENTAL METHODS

2.1 Insect strains

A susceptible strain of *B. tabaci* (biotype B), used in all bioassays, was collected in 1987 from cotton fields and thereafter reared in isolation, with no exposure to any insecticides. The whiteflies were reared on cotton seedlings (*Gossypium hirsutum* L. cv. Acala) under standard laboratory conditions of 26 ± 2 °C and a 14 : 10 h light : dark photoperiod. Other whitefly populations used in this study were collected in different locations in Israel.

2.2 Insecticides

Spiromesifen was applied as a 240 g L^{-1} SC (Oberon® 240 SC; Bayer CropScience, Germany).

2.3 Egg and instar bioassays

Cotton seedlings infested with first instars or with 0–2-day-old eggs were dipped in various concentrations of the compound or in water as a control. Cumulative nymphal mortality (expressed as suppression of pupation) was determined 10 and 18 days after application of the compound. Foliar application on eggs was used for spiromesifen resistance monitoring in Israel.

2.4 Adult bioassays

A cotton stem with two true leaves was placed in a plastic vial containing water to prevent desiccation and covered with parafilm. Leaves were dipped for 20 s in 5 mg AI L^{-1} (aqueous concentration) of the formulated compound, or in deionized water (control). After air drying, vials with the treated leaves were put into glass jars and 10–20 whiteflies (females or males, sexed under a stereoscope) were introduced into each. Adult mortality was determined after different days of exposure to treated leaves. Bioassays for each exposure period were performed in five replicates (i.e. five jars).

2.5 Effect on fecundity and fertility

Mated female whiteflies (3–5 days old) were exposed to cotton leaves treated with various concentrations of the tested compound as described above. After different pre-exposure periods, the whiteflies were collected and transferred to untreated cotton plants. Each leaf was infested with 5–10 females confined in clip cages for 24 h oviposition. The number of eggs per female (fecundity) was then determined. The percentage of hatched eggs (fertility) was determined after 8 days.

2.6 Translaminar application

Leaves of cotton seedlings infested with first instars on their lower surfaces were treated with a paint brush on only their upper surfaces with various concentrations of spiromesifen (at an application volume of 50 µL of solution per leaf). Cumulative nymphal mortality (expressed as suppression of pupation) was determined 10 days after treatment at the pupation stage.

2.7 Systemic application

For stem application, stems with two true leaves infested with first instars were placed separately in plastic vials containing various concentrations of the compound or water as a control and covered with parafilm. Nymphal mortality was determined 4 and 7 days later. For soil application, leaves of potted cotton seedlings were exposed to 10–15 *B. tabaci* females confined in clip cages for 24 h oviposition. The number of first instars was counted after 8 days. Soil of potted seedlings infested with *B. tabaci* nymphs was drenched with various spiromesifen concentrations, then kept under standard laboratory conditions as described above. The soil in all tests (250 mL pots) was prepared from 70% peat and 30% tuff. Aqueous solutions (25 mL) containing various spiromesifen concentrations were applied to the soil. The effect of spiromesifen on nymphal mortality (expressed as suppression of pupation) was determined 10 days after application.

2.8 Residual activity

Cotton seedlings were treated with various spiromesifen concentrations. The treated plants were periodically exposed to *B. tabaci* females confined in clip cages for 24 h oviposition, and the number of eggs laid was then counted. Nymphal mortality, expressed as suppression of pupation, was determined after 18 days. Each bioassay was performed with four concentrations, 5–10 replicates each.

2.9 Light and scanning electron microscopy (SEM)

For light microscopy, eggs were separated from the leaves and submerged in 80% glycerol on glass slides. They were then covered with coverslips and viewed under a Leica compound microscope. A total of 87 eggs laid by treated females and 42 eggs laid by untreated females were processed for light microscopy. A total of 25 treated females and over 100 eggs laid by treated females and 15 untreated females and 50 eggs laid by untreated females were processed for SEM analysis. Eggs and adult females were treated and viewed as previously described.[18]

2.10 Fluorescence *in situ* hybridization (FISH)

FISH of eggs and nymphs was performed exactly as described by Gottlieb *et al*.[19] with the symbiont-specific 16S rRNA DNA probes given in Table 2 of that reference. Whole-mounting confocal microscopy, reproducibility and controls were performed exactly as described by Gottlieb *et al*.[19]

2.11 4′,6-Diamidino-2-phenylindole (DAPI) staining of ovaries

Ovaries were dissected from three-day-old mated female whiteflies, fixed in 4% formaldehyde for 1 h and washed 3 times in 1× phosphate-buffered saline (PBS). They were then incubated in 0.1 mg mL^{-1} DAPI in 1× PBS solution for 15 min, washed 3 times, 5 min each with 1× PBS, submerged in 80% glycerol on a microscopic glass slide, covered with a cover slip and viewed under a Leica fluorescent compound microscope.

2.12 Data analysis

Probit analyses of the concentration-dependent mortality data were performed using POLO-PC,[20] after correction with Abbott's formula.[21] Failure of 95% CL (confidence limits) to overlap at a particular lethal concentration indicated a significant difference. All results comparing differences in adult mortality, fertility and

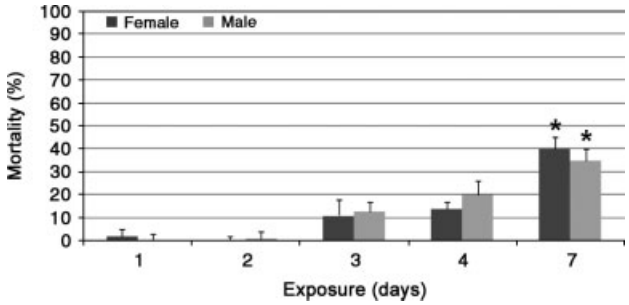


Figure 1. Corrected mortality rates of *Bemisia tabaci* males and females after exposure to 5 mg L^{-1} spiromesifen for 1–7 days. Asterisks indicate significantly different mortality rates after 7 days of exposure in both males and females.

fecundity and the number of ovarioles to those of the control were statistically analysed using a paired *t*-test with $\alpha = 0.05$. Error bars in all graphs represent the standard error of the mean (SEM).

3 RESULTS

3.1 Effect of spiromesifen on adult mortality

Male and female whitefly mortality rates were tested using the bioassays described in Section 2. Figure 1 shows that, although spiromesifen affected both males and females (significantly after 7 days), only 40–50% mortality was obtained. This result indicated that spiromesifen is moderately effective against adults. The results further indicated that males and females are equally affected, as can be noted after 7 days of spiromesifen exposure (Fig. 1).

3.2 Effect of spiromesifen on egg and nymphal stages

Spiromesifen caused considerable mortality rates when first nymphs (LC_{50} = 0.5 mg AI L^{-1}) and eggs (LC_{50} = 2.6 mg AI L^{-1}) were treated (Fig. 2). The effect after first nymph treatment was stronger than the effect after egg treatment.

3.3 Effect of spiromesifen on fertility and fecundity

After 1 day of exposure, the number of eggs laid by treated females, presented as the number of eggs laid by one female after a 24 h period on average, was not significantly different from controls (Fig. 3). However, significant reductions in fecundity were observed after 2 days exposure to leaves treated with the highest

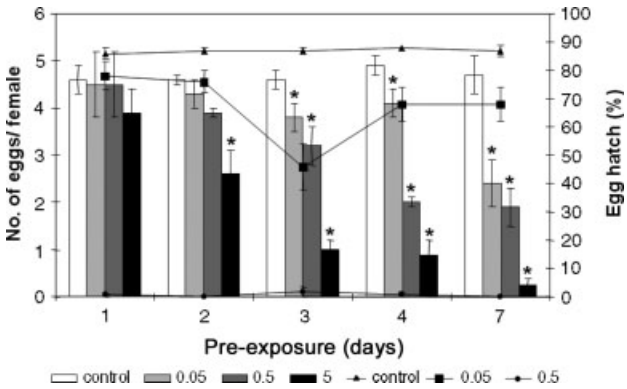
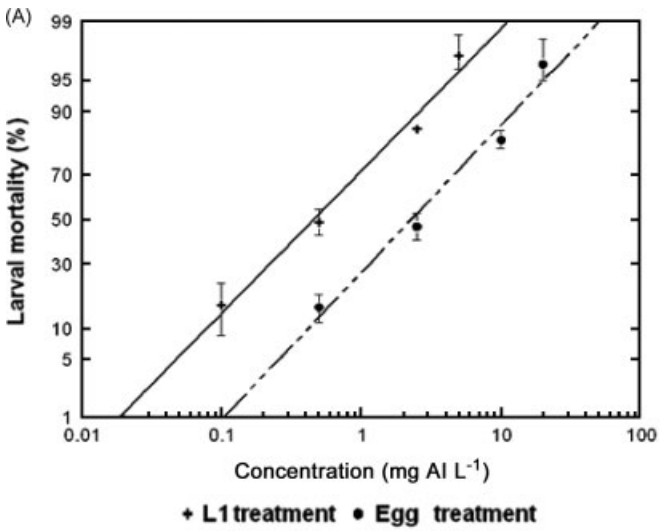


Figure 3. Effect of different concentrations (mg L^{-1}) of spiromesifen on the number of eggs laid by a single female (fecundity, bars) and egg hatch (fertility, lines). Asterisks indicate significant differences.



(B)

Treated stage	n^a	Slope ± SEM	LC_{50}^b (95% CL)	LC_{90}^b (95% CL)
L1	630	1.55±0.14	0.5 (0.2–0.8)	3.2 (1.9–9.1)
Eggs	2039	1.77±0.11	2.6 (0.4–4.9)	14 (8–53)

[a] *n* = total number of individuals tested from each stage.
[b] LC_{50} and LC_{90} values are in mg AI L^{-1}.

Figure 2. (A) Log concentration–response curves (on a probit scale) for the effect of spiromesifen on *Bemisia tabaci* egg and first-instar mortality; (B) parameters for the effect of spiromesifen applied on eggs and first nymphs.

concentration of spiromesifen (5 mg AI L^{-1}), and after 3–7 days of exposure to the three concentrations tested (0.05, 0.5 and 5 mg AI L^{-1}). These results suggested strong and rapid action of spiromesifen on female fecundity (Fig. 3).

The effect of two different concentrations (0.05 and 0.5 mg AI L^{-1}) of spiromesifen on *B. tabaci* fertility was compared with the control. While egg hatch in the controls reached up to 90% across 7 days, almost no egg hatch was observed when the females were treated with 0.5 mg AI L^{-1} spiromesifen, from the very first day of treatment. Egg hatch reached between 50 and 70% when females were treated with 0.05 mg L^{-1} spiromesifen.

3.4 Systemic, translaminar and residual activity of spiromesifen

Figure 4 demonstrates that, when spiromesifen is acquired by the plant in a systemic manner (through the stems), the effect is similar to foliar application (compare LC_{50} values in Fig. 4B for cut seedlings treated for 7 days and foliar treatment). When spiromesifen was applied to the soil, it showed moderate activity (Fig. 4B).

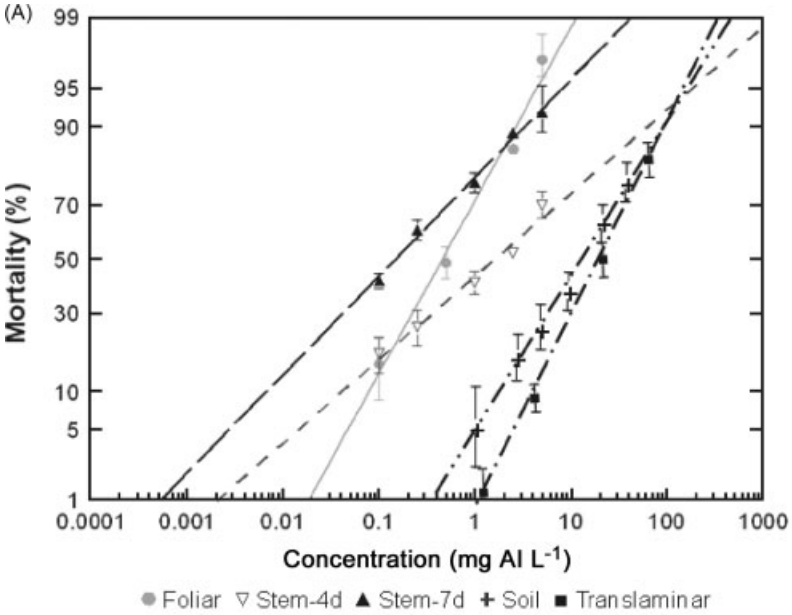
Translaminar activity of spiromesifen was also moderate (Fig. 4), indicating some difficulty in transporting the material from one side of the leaf to the other.

The residual activity of spiromesifen was measured by applying the compound at different concentrations, as described in Section 2.8. The best results were obtained with a concentration of 50 mg AI L^{-1} for a 21 day period: activity decreased from about 100% mortality to 80% mortality after 2 weeks, and to 60% mortality after 3 weeks (Fig. 5).

3.5 Effect of spiromesifen on ovary, egg and bacteriome morphology

The experiments performed to this point indicated that spiromesifen has a strong effect on egg development, as demonstrated by the lower number of eggs laid after treatment. Eggs laid by treated females were therefore examined under the dissecting microscope, and clear abnormalities of the chorion were observed. Moreover, 72-h-old eggs laid by females treated with 5 mg L^{-1} spiromesifen and viewed under a light microscope showed abnormal internal structure and clearing of the content and structures

(A)



(B)

Application	n^a	Slope ± SEM	$LC_{50}{}^b$ (95% CL)	$LC_{90}{}^b$ (95% CL)
Foliar	630	1.55±0.14	0.5 (0.2–0.8) **b**	3.2 (1.9–9.1) **a**
Systemic stem – 4 days	677	0.7±0.13	1.6 (0.9–3.2) **c**	105 (27–1779) **b**
Systemic stem – 7 days	696	0.98±0.12	0.14 (0.08–0.2) **a**	3 (2–6) **a**
Soil	789	1.49±0.20	13 (10–17) **d**	96 (61–204) **b**
Translaminar	793	1.66±0.19	18 (14–23) **d**	106 (76–169) **b**

[a] n = total number of individuals tested for each application.
[b] LC_{50} and LC_{90} values are in mg AI L^{-1}.
Different letters indicate significant differences within columns.

Figure 4. (A) Log concentration–response curves (on a probit scale) for the effect of different methods of spiromesifen application (systemically through the stem for 4 and 7 days, systemically following application to the soil, translaminar and foliar) on mortality of a *Bemisia tabaci* susceptible line (Ssc); (B) parameters for the effect of spiromesifen applied by different methods as indicated in A.

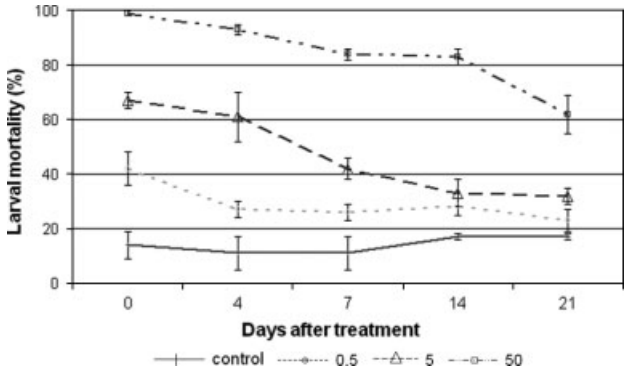


Figure 5. Residual activity of spiromesifen at different concentrations (mg L^{-1}) on *Bemisia tabaci* over 21 days after treatment.

inside the egg (Figs 6A and B). The bacteriome in the treated eggs (Fig. 6B) did not move to the centre, as it does in normal eggs (Fig. 6A). SEM analysis showed rough and abnormal development of the chorion (Fig. 6D) of eggs laid by treated females, compared with the smooth chorion of the control eggs (Fig. 6C). The abnormal eggs were sometimes properly laid, but in many cases they were stuck in the female ovipositor (Fig. 6F). The abnormal eggs that were successfully laid were improperly inserted into the leaf surface owing to an abnormal and malformed egg pedicel (Figs 6G and H). Pedicels of eggs that are normally laid are not usually visible because they are completely inserted into the leaf tissue.

Special attention was given to the effect of spiromesifen on bacteriome development. Bacteriomes in *B. tabaci* harbour the primary symbiont *Portiera*, which occupies most of the bacteriome space, and the secondary symbionts *Hamiltonalla* in the B biotype and *Arsenophonus* and *Wolbachia* in the Q biotype.[19,22] Both biotypes harbour *Rickettsia*, which is usually scattered outside the bacteriome.[19] The effect of spiromesifen treatment on the bacteriome in eggs and nymphal stages was described following FISH of the primary symbiont *Portiera* and the secondary symbiont *Rickettsia*, which localizes outside the bacteriome (Fig. 7). In 24-h-old untreated eggs the bacteriome is proximally localized adjacent to the pedicel (Fig. 7A). Treating 24-h-old eggs with 0.5, 5 or 50 mg L^{-1} spiromesifen (Figs 7B, C and D respectively) caused arrest of movement and partial degradation of the bacteriome. In 72-h-old untreated eggs (Fig. 7E) the bacteriome is distally localized relative to the pedicel and properly moves towards the egg centre, while in treated eggs with 0.5, 5 and 50 mg L^{-1} spiromesifen (Figs 7F, G and H respectively) the bacteriome is completely degraded and arrested proximally to the pedicel. *Rickettsia* distribution was not affected when eggs were treated with 0.5 and 5 mg L^{-1} spiromesifen (Figs 7B, C, F and G, blue), while their distribution was considerably reduced after treatment with 50 mg L^{-1} (Figs 7D and H). High spiromesifen concentrations also caused changes in the egg shape and size (Figs 7D and H). To support the hypothesis that the effects seen on the bacteriome are specific following spiromesifen treatment, eggs were treated with a lethal concentration of 200 mg L^{-1} pyriproxyfen, an insecticide that mimics the juvenile hormone action and acts transovarially, similarly to spiromesifen. The results (Fig. 7I) showed that pyriproxyfen treatment did not cause any effect on the bacteriome morphology and movement, suggesting a specific effect of spiromesifen on the bacteriome. Spiromesifen further caused developmental effects on

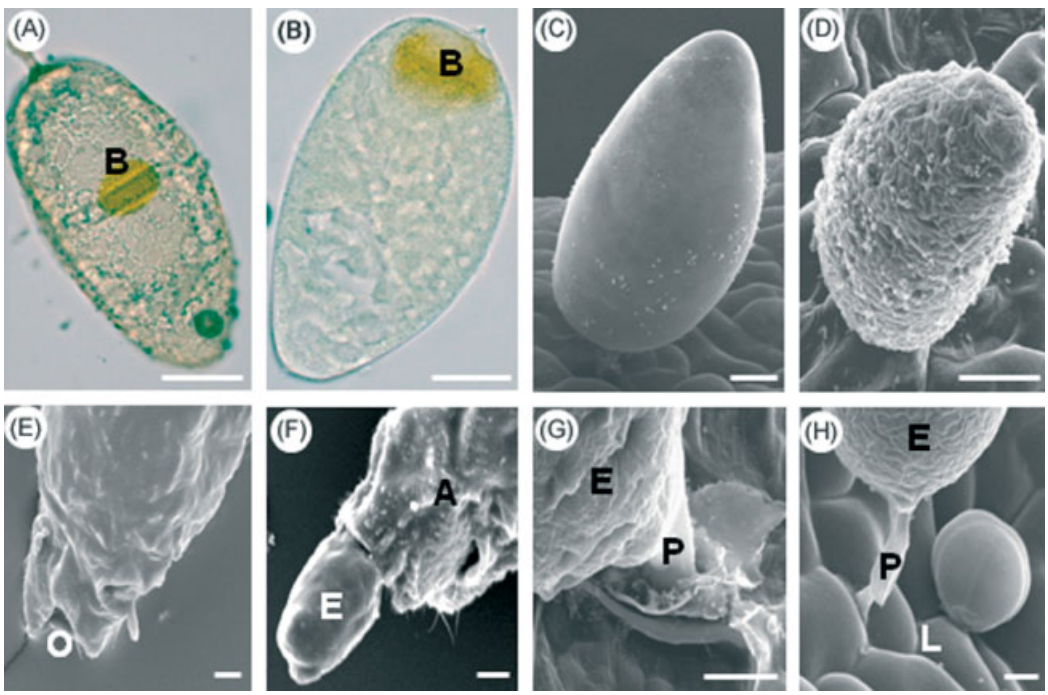


Figure 6. Effect of spiromesifen on egg morphology and the oviposition process: (A) light micrograph of a *Bemisia tabaci* egg laid by an untreated female; (B) light micrograph of an egg laid by a female treated with 5 mg L^{-1} spiromesifen, showing defects in bacteriome movement; (C) SEM micrograph of a normal egg as in (A); (D) SEM micrograph of an abnormal egg as in (B), showing defects in the external morphology; (E) tip of the abdomen of an untreated female; (F) tip of the abdomen of a female treated with 5 mg L^{-1} spiromesifen, showing an abnormal egg stuck in the ovipositor; (G) and (H) eggs laid by treated females, showing abnormal insertions of the pedicel into the leaf. B, bacteriome; O, ovipositor; E, egg; A, abdomen; P, pedicel; L, leaf. Bars in (A) and (B) are 70 μm, in (C), (D), (E) and (F) they are 25 μm and in (G) and (H) they are 10 μm.

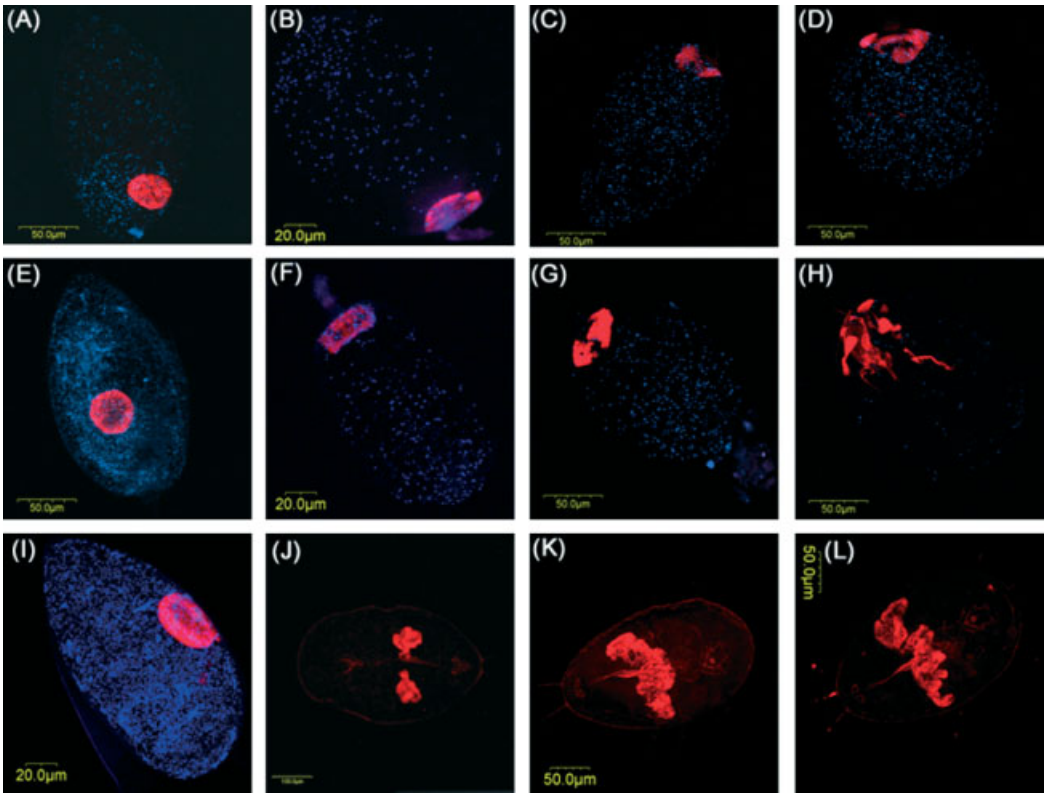


Figure 7. FISH of *Bemisia tabaci* eggs using a *Portiera*-specific probe (red) and *Rickettsia*-specific probe (blue), and *B. tabaci* nymphs using only a *Portiera*-specific probe (red): (A) 24-h-old untreated egg; (B)–(D) 24-h-old eggs treated with 0.5, 5 and 50 mg L^{-1} spiromesifen; (E) 72-h-old untreated egg; (F)–(H) 72-h-old eggs treated with 0.5, 5 and 50 mg L^{-1} spiromesifen; (I) 72-h-old egg treated with 200 mg L^{-1} pyriproxyfen; (J) untreated second-stage nymph; (K) and (L) second-stage nymphs treated with 2 mg L^{-1} spiromesifen.

bacteriomes in second nymphal stages after treatment with 2 mg AI L^{-1}. FISH analysis of *Portiera* showed that the two bacteriome bodies never separated (Figs 7K and L) as seen in untreated nymphs (Fig. 7J), and they were developmentally disrupted and unevenly formed.

The lower number of eggs laid by the treated females was also accompanied with abortion of some of the immature eggs in the female before they were laid. This was evidenced by the number of ovarioles in each ovary dissected from females treated with 5 mg L^{-1} spiromesifen and from untreated females. A normal untreated female carries two ovaries; each has 10–13 ovarioles. Figure 8A shows one ovary with ten ovarioles, dissected from an untreated female and stained with DAPI. While the untreated females averaged 22.5 ovarioles in two ovaries per female (the range of the number of ovarioles in untreated females was 19–24 because sometimes 1–2 ovarioles were miscalculated on account of being too small or lost while the specimens were being processed), the treated females had an average of 6.1 ovarioles in two ovaries (Fig. 8B). This result may explain the significant decrease in fecundity in treated females.

3.6 Monitoring spiromesifen resistance status in Israel and cross-resistance with major insecticides

Spiromesifen was introduced in Israel in 2004, and since then it has been registered for use with several field crops, vegetables and ornamentals. The annual resistance monitoring programme conducted in Israel included spiromesifen in the 2006–2007 seasons. The present results indicated that no resistance to spiromesifen had evolved among populations collected across Israel (Fig. 9A). Treating laboratory-raised populations of *B. tabaci* biotypes B and Q that are resistant to major insecticides, including neonicotinoids, pyriproxyfen and diafenthiuron, with spiromesifen showed no cross-resistance to these compounds (Fig. 9B). It should be noted that, unlike the bioassays for translaminar, soil and systemic application, in the resistance monitoring and cross-resistance bioassays, plants with eggs were dipped into the compound because eggs are easier to work with. In addition, for monitoring experiments, the speed with which the compound reaches the target site is not important as long as the same application method is used. In the foliar, systemic, soil and translaminar tests, which are different application methods, the speed with which the compound reaches the target site is different, and thus it is important to use the most susceptible stage in order to see the effect as quickly as possible. For example, in the foliar application, the compound reaches the insect faster than in the systemic application, and thus, in order to see the maximum effect, it is preferable to use the most sensitive stage.

4 DISCUSSION

This study demonstrates the variability among *B. tabaci* developmental stages in their susceptibility to spiromesifen. The mode of action of this compound is novel (inhibition of lipid synthesis), and it has a very specific and strong effect on the egg and nymphal stages, as shown here (Fig. 2) and in other studies,[15–17] while adults and late nymphal stages are only moderately affected (Fig. 1). A recent study that tested the efficacy of spiromesifen

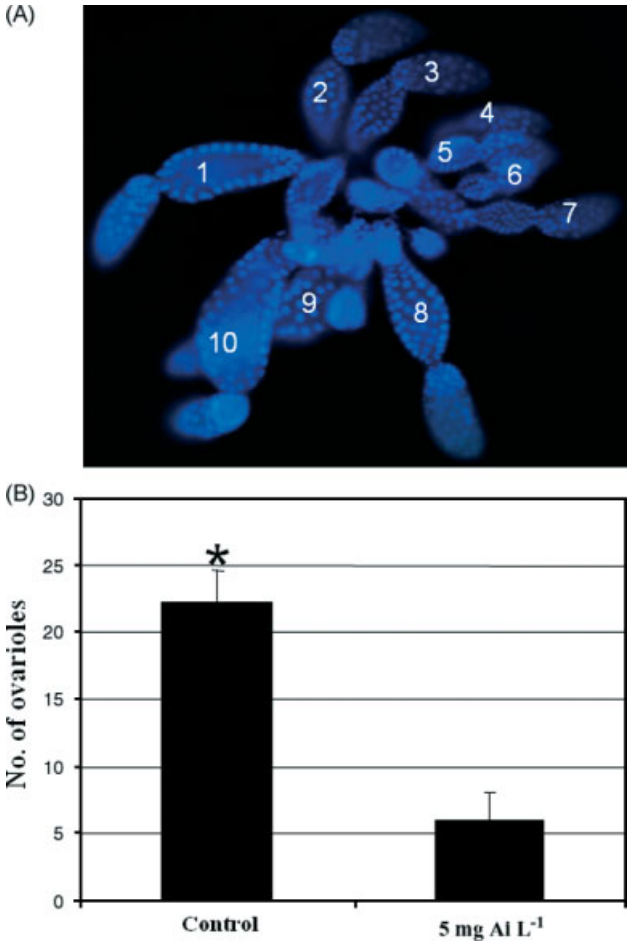


Figure 8. (A) DAPI staining of untreated *Bemisia tabaci* ovary showing ten ovarioles; (B) average number of ovarioles counted in untreated females and females treated with 5 mg L^{-1} spiromesifen. Asterisk indicates a significant difference.

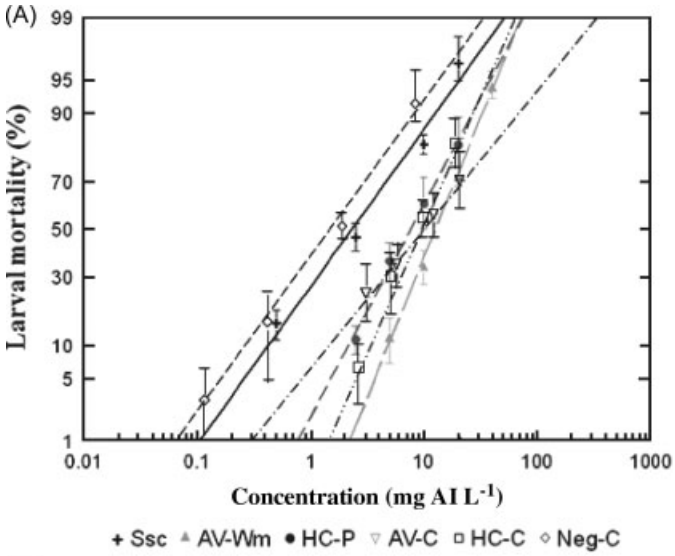
on field-collected *B. tabaci* populations in Arizona and California, and on laboratory resistant strains, showed results very similar to those obtained in the present work.[23] This study showed that the first-instar stage was the most sensitive stage among all nymphal stages, and LC_{50} values for this stage ranged from 0.2 to 6 mg AI L^{-1}, while the present results show a range of 0.2–0.8 mg AI L^{-1}.

One of the important findings of the present study was the considerable effect of spiromesifen on juvenile stages of *B. tabaci* after treating adult females with the compound, suggesting a strong transovarial effect, which also led to a powerful effect on fecundity and fertility (Fig. 3). Similarly to previous work, the transovarial effect was observed on newly oviposited eggs, suggesting a strong effect during the oogenesis process in the female.[15] The transovarial effect was evidenced by the morphological effects seen on newly laid eggs, by the reduction in fecundity owing to the effect on developing oocytes inside the female and by the number of ovarioles in treated females, which was significantly reduced, suggesting abortion of some of the eggs inside the female (Fig. 8). Many developmental defects were observed in the eggs immediately after they were deposited by treated females, suggesting that spiromesifen acts on an important step during oogenesis that requires the deposition of lipids. Light microscopy and SEM showed defects in the egg chorion and in pedicel insertion into the leaf, internal developmental defects, such as those of the bacteriome, and problems in the oviposition process (Figs 6 and 7). The observed egg defects were instrumental in causing oviposition problems, which may explain the lower number of eggs laid by the treated females. Eggs oviposited with defects never hatched, but not all eggs laid by treated females had defects, suggesting dose dependency of the effects. This is the first report in which developmental defects have been associated with spiromesifen. The reduction in fecundity was not due to females being killed by the compound, because only live females were taken for egg-laying experiments after the treatment. Furthermore, the present work shows that these effects are obtained with treatment of both the egg and the first nymphal stage. The egg stage was found to be less sensitive than the first nymphal stage. It is not known whether spiromesifen penetrates the egg, or whether the smaller effect seen on eggs compared with first instars is because the compound does not penetrate the egg at all and the effect seen is the residual effect on first instars, or whether very little of the compound treated to eggs reaches the target site (because of the egg chorion) and thus a reduced effect is seen on eggs compared with first instars. The abnormal eggs that developed inside the female might be a factor in female mortality, thereby conferring an additional and an indirect effect of spiromesifen.

Spiromesifen is applied to the foliage. Its biological profile was therefore constructed on the basis of foliar application,[15] and it was considered to be a non-systemic compound.[15] The present results show, however, that this compound has appreciable systemic activity when applied directly to the soil, and is more active when acquired by the seedlings through their stems (Fig. 4), an effect that depends on exposure duration. Although the time in which the compound reaches the target insect is different in the application methods tested here, the authors believe the higher LC_{50} values obtained in the soil and translaminar applications to be due to the interaction between the compound and the soil particles, or the entrance of the compound to the roots in the soil application, and to the difficulty of the translaminary passage of the compound from one side of the leaf to the other. The speed of spiromesifen movement in soil and plant tissues probably depends on the physical and chemical properties of the compound, which could not be controlled in the present experiments. The results of these two application methods might also be due to the final concentration that reaches the targeted insect, which is lower than in the foliar and systemic applications, or to the fact that the time needed for the same concentration of the compound to reach the target insect is longer than in the foliar and systemic applications, and was outside the time intervals used in the present experiments. In practice, foliar application in the field is still the most relevant application method, but systemic application may be of practical interest for exported products such as cut flowers,[24] which can be treated with the compound to avoid inoculation with *B. tabaci* before export.

The residual activity of spiromesifen was also studied: treatment with 50 mg AI L^{-1} was still effective after 14 days, with more than 80% nymphal mortality. These values are similar to those previously reported,[16] i.e. 90% nymphal mortality after 14 days following treatment with 40 mg L^{-1} spiromesifen.

Spiromesifen was introduced in Israel in 2004 and is used with field and vegetable crops. Monitoring the response of field-collected *B. tabaci* populations from these crops showed no development of resistance, and the response curves of several



(B)

Strain [a]	*n* [b]	Slope ± SEM	LC_{50} [c] (95% CL)	LC_{90} [c] (95% CL)	RR_{50} [d]	RR_{90}
Ssc (1)	2039	1.77±0.11	2.6 (0.4–4.9)	14 (8–53)	1	1
Tgt-R-Q (3500)	3584	1.93±0.10	1.1 (1.0–1.3)	5.2 (4.8–5.9)	0.4	0.4
Mospilan-R-Q (200)	2492	3.33±0.16	3 (2–4)	8 (6–11)	1	0.6
Act-R-Q (1000)	3405	1.85±0.08	6 (4–7)	49 (37–72)	2	4
Act-R-B (100)	1232	1.63±0.14	0.8 (0.3–1.2)	5 (3–14)	0.3	0.4

[a] strain = resistance ratio to selected strain.
[b] *n* = total number of individuals tested for each material.
[c] LC_{50} and LC_{90} values are in mg AI L^{-1}.
[d] RR (resistance ratio) = LC field population/LC susceptible strain.

Figure 9. (A) Log concentration–response curves (on a probit scale) for *Bemisia tabaci* resistance monitoring of spiromesifen in Israel in early 2006–2007 seasons. Av-Wm, populations collected from the Ayalon Valley area (Central Israel) on watermelon; Av-C, populations collected from the Ayalon Valley area on cotton; Neg-C, populations collected from the Negev area (Southern Israel) on cotton; HC-P, populations collected from the Carmel coastal area (Northern Israel) on peppers; HC-C, populations collected from the Carmel coastal area on cotton; Ssc, laboratory-raised susceptible line. (B) Cross-resistance of spiromesifen to selected *B. tabaci* strains resistant to major insecticides including neonicotinoids, pyriproxyfen and diafenthiuron.

of the collected populations were similar to that of the present susceptible laboratory strain (Fig. 9A). These results demonstrate similarly high sensitivity to spiromesifen among populations across Israel, and are supported by the high sensitivity to spiromesifen found in cross-resistance tests conducted among several *B. tabaci* strains resistant to widely used insecticides, including pyriproxyfen, thiamethoxam and acetamiprid (Fig. 9B). The cross-resistance results are similar to previous cross-resistance results obtained on *B. tabaci* B and Q biotype populations collected in California and Arizona.[23] Furthermore, previous reports demonstrated the extreme effectiveness of spiromesifen against pyriproxyfen-resistant whiteflies.[15] These results suggest that spiromesifen can be used as a new and valuable tool in whitefly resistance management when combined with neonicotinoid (thiamethoxam and acetamiprid) insecticides. Because *B. tabaci* has developed resistance to organophosphates, pyrethroids, some insect growth regulators and some neonicotinoid insecticides,[3–5,25] the unique mode of action of spiromesifen may give it an important role in resistance management programmes.

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REFERENCES

1 Brown JK, Frohlich DR and Rosell RC, The sweet-potato or silverleaf whiteflies – biotypes of *Bemisia tabaci* or a species complex. *Ann Rev Entomol* **40**:511–534 (1995).
2 Inbar M and Gerling D, Plant-mediated interactions between whiteflies, herbivores, and natural enemies. *Ann Rev Entomol* **53**:431–448 (2008).
3 Cahill M, Denholm I, Byrne FJ and Devonshire AL, Insecticide resistance in *Bemisia tabaci*: current status and implications for management. *Proc Brighton Crop Prot Conf – Pests and Diseases, Vol. 3*, British Crop Protection Council, Farnham, UK, pp. 75–80 (1996).
4 Cahill M, Gorman K, Day S, Denholm I, Elbert A and Nauen R, Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull Entomol Res* **86**:343–349 (1996).
5 Palumbo JC, Horowitz AR and Prabhaker N, Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Prot* **20**:739–765 (2001).
6 Horowitz AR and Ishaaya I, Biorational insecticides – mechanisms, selectivity and importance in pest management programs, in *Insect Pest Management – Field and Protected Crops*, ed. by Horowitz AR and Ishaaya I. Springer, Berlin–Heidelberg–New York, pp. 1–28 (2004).
7 Ishaaya I, Kontsedalov S and Horowitz AR, Biorational insecticides: mechanism and cross-resistance. *Arch Insect Biochem Physiol* **58**:192–199 (2005).

8 Ishaaya I and Horowitz AR, In focus: IPM using novel insecticides and other approaches. *Pest Manag Sci* **63**:729 (2007).
9 Denholm I, Cahill M, Byrne FJ and Devonshire AL, Progress with documenting and combating insecticide resistance in *Bemisia*, in *Bemisia: 1995 Taxonomy, Biology, Damage, Control and Management*, ed. by Gerling D and Mayer RT. Intercept Press, Andover, UK, pp. 577–603 (1995).
10 Liu TX and Meister C, Managing *Bemisia tabaci* on spring melon with insect growth regulators, entomopathogens and imidacloprid in south Texas. *Subtrop Plant Sci* **53**:44–48 (2001).
11 Liu TX, Evaluating two nicotinyl insecticides, imidacloprid and thiamethoxam, against silverleaf whitefly on spring melons in South Texas. *Subtrop Plant Sci* **54**:41–43 (2002).
12 Horowitz AR, Kontsedalov S, Denholm I and Ishaaya I, Dynamics of insecticide resistance in *Bemisia tabaci*: a case study with the insect growth regulator pyriproxyfen. *Pest Manag Sci* **58**:1096–1100 (2002).
13 Ishaaya I, Kontsedalov S and Horowitz AR, Novaluron (Rimon), a novel IGR: potency and cross-resistance. *Arch Insect Biochem Physiol* **54**:157–164 (2003).
14 Bretschneider T, Benet-Buchhol J, Fischer R and Nauen R, Spirodiclofen and spiromesifen – novel acaricidal and insecticidal tetronic acid derivatives with a new mode of action. *Chimia* **57**:697–701 (2003).
15 Nauen R, Bretschneider T, Elbert A, Fischer R and Tieman R, Spirodiclofen and spiromesifen. *Pestic Outlook* **14**:243–246 (2003).
16 Nauen R, Schnorbach HJ and Elbert A, The biological profile of spiromesifen (Oberon) – a new tetronic acid insecticide/acaricide. *Pflanzenschutz-Nachrichten Bayer* **58**:417–440 (2005).
17 Nauen R and Konanz S, Spiromesifen as a new chemical option for resistance management in whiteflies and spider mites. *Pflanzenschutz-Nachrichten Bayer* **58**:485–502 (2005).
18 Ghanim M, Rosell RC, Campbell LR, Czosnek H, Brown JK and Ullman DE, Digestive, salivary, and reproductive organs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) B type. *J Morphol* **248**:22–40 (2001).
19 Gottlieb Y, Ghanim M, Gueguen G, Kontsedalov S, Vavre F, Fleury F, *et al*, Inherited intracellular ecosystem: symbiotic bacteria share bacteriocytes in whiteflies. *FASEB J* **22**:2591–2599 (2008).
20 *POLO-PC. A User's Guide to Probit or Logit Analysis.* LeOra Software, Berkeley, CA (1987).
21 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
22 Chiel E, Gottlieb Y, Inbar M, Zchori-Fein E and Ghanim M, Distribution of secondary symbionts in Israeli populations of *Bemisia tabaci*. *Bull Entomol Res* **97**:407–413 (2007).
23 Prabhaker N, Castle SJ, Buckelew L and Toscano NC, Baseline susceptibility of *Bemisia tabaci* B biotype (Hemiptera: Aleyrodidae) populations from California and Arizona to spiromesifen. *J Econ Entomol* **101**:174–181 (2008).
24 Hansen JD, Hara AH and Tenbrink VL, Insecticidal dips for disinfesting commercial tropical cut flowers and foliage. *Trop Pest Manag* **38**:245–249 (1992).
25 Prabhaker N, Toscano NC, Castle SJ and Henneberry TJ, Selection for imidacloprid resistance in silverleaf whiteflies from the Imperial Valley and development of a hydroponic bioassay for resistance monitoring. *Pestic Sci* **51**:419–428 (1997).

