

Efficacy of Spiromesifen Against Greenhouse Whitefly (Homoptera: Aleyrodidae) on Strawberry

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Abstract. Spiromesifen is a novel insecticide (belonging to the new chemical class of spirocyclic phenyl-substituted tetrone acids) with a unique mode of action. Laboratory and field experiments were conducted to test the efficacy of this insecticide against the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) on strawberry, *Fragaria ananassa* (L.). Laboratory experiments showed that spiromesifen at 0.5 and 1.0 $\mu\text{g}\cdot\text{mL}^{-1}$ a.i. inhibited egg hatching by 80% and 100%, respectively, whereas at concentrations of 3.1, 3.0, and 10.0 $\mu\text{g}\cdot\text{mL}^{-1}$ a.i., this insecticide, respectively, killed 100% of the first, second, and third instar nymphs. Much lower toxicity to adults was observed. Field trials revealed that application of spiromesifen reduced the whitefly egg numbers by 61% to 80% from 2 to 3 weeks posttreatment in comparison with the pyriproxyfen treatment, whereas the application lowered the egg numbers by 34% to 73% from 2 to 5 weeks posttreatment compared with the buprofezin treatment. In comparison with pyriproxyfen treatment, spiromesifen application decreased the numbers of immature whiteflies by 29% to 92% from 1 to 6 weeks posttreatment. The effect of spiromesifen on reduction of immatures was similar to that of buprofezin. Also, the efficacy of spiromesifen on suppression of adult numbers was comparable to that of pyriproxyfen or buprofezin. Spiromesifen shows promise for inclusion in integrated greenhouse whitefly management programs and insecticide resistance management programs on strawberry.

California is a world leader in strawberry production. Since the late 1990s, strawberry production has been threatened by a major insect pest, the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) (Bi et al., 2002a, 2002b, 2002c). This pest removes a large amount of phloem sap from strawberry plants, resulting in decreased fruit yield and reduced fruit quality through reduction of glucose, citric acid, and vitamin C (Bi and Toscano, unpublished data; McKee and Zalom, 2007). In addition, this pest decreases the marketable value of strawberry fruits through honeydew and the associated sooty mold contaminations and transfers plant virus diseases (Bi et al., 2002a, 2002b, 2002c; F. G. Zalom, pers. comm., 2005). Control of this pest in California has been heavily dependent on chemical insecticides, including neonicotinoids, insect growth regulators, and some conventional insecticides.

Imidacloprid is a neonicotinoid insecticide, which acts on acetylcholine receptors in the insect central nervous system with sys-

temic properties and long residual activity against sucking insects such as whiteflies (Kagabu, 1999; Ware, 2000; Yamamoto, 1999). The residual activity in strawberry against the greenhouse whitefly is over 2 months after soil application (Bi et al., 2002a, 2002b). Since its first emergency registration in 1999 under U.S. Environmental Protection Agency Section 18 exemption on strawberry in California, imidacloprid (trade name Admire) has been used most intensively to control the whiteflies. Pyriproxyfen is an insect growth regulator affecting the hormonal balance in insects and resulting in a strong suppression of embryogenesis and adult formation (Ishaaya and Horowitz, 1998). Pyriproxyfen (trade name Esteem) is very effective in inhibiting the whitefly egg hatching after foliar spray and has been registered on strawberry in California since 2003 (Bi et al., 2002a, 2002b). The use of imidacloprid at transplanting in fall followed by the application of pyriproxyfen in early spring provides the greatest control of the whiteflies on strawberry (McKee and Zalom, 2007). Commonly used conventional insecticides against whiteflies on strawberry include endosulfan (chlorinated hydrocarbon), chlorpyrifos and malathion (organophosphate), methomyl (carbamate), bifenthrin and fenprothrin (pyrethroid) (Bi and Toscano, 2007). The residual activities of these insecticides against the greenhouse whitefly on strawberry are ≈ 1 week. Application of these insecticides is only recommended to suppress high adult whitefly

populations in mid or late season after the efficacy of imidacloprid or pyriproxyfen applied at transplanting or early season diminishes (Liu and Meister, 2001; Polumbo et al., 2001).

Extensive reliance on chemical insecticides for whitefly control has resulted in whitefly resistance to almost all major classes of conventional insecticides throughout the world (Omer et al., 1992; Polumbo et al., 2001; Wardlaw et al., 1972, 1975, 1976; Zou and Zheng, 1988). We recently detected significant tolerance/resistance of the greenhouse whitefly to imidacloprid in strawberry in California (Bi and Toscano, 2007). Our results strongly emphasize the need to develop resistance management strategies in the region. Introduction of novel insecticides with distinct modes of action into the current whitefly control program is a valuable tactic for resistance management (Denholm et al., 2002; Liu, 2004).

Spiromesifen is a novel insecticide and acaricide belonging to the new chemical class of spirocyclic phenyl-substituted tetrone acids (Nauen et al., 2002). This compound acts on interfering with insect/mite lipid biosynthesis (Nauen et al., 2002). Spiromesifen is especially active against whiteflies (*Bemisia* spp. and *Trialeurodes* spp.) and spider mites (*Tetranychus* spp.) in several cropping systems, including cotton (*Gossypium hirsutum* L.), vegetables, and ornamentals (Liu, 2004; Nauen et al., 2002; Polumbo, 2004). The present study was initiated to test the efficacy of spiromesifen against the greenhouse whitefly on strawberry under both laboratory and field conditions.

Materials and Methods

Laboratory experiment

Plants, insects, and insecticides. Strawberry bare-root seedlings (cv. Camarosa from Sierra-Cascade Nursery, Susanville, Calif.) were planted in 2.6-L pots filled with a 1.3:1 sand: peatmoss mixture (by volume) in environmental growth chambers. Plants used in the experiments were at the three to five trifoliate stage. Plants were watered every 2 d. At the time of planting, 5 g of Osmocote fertilizer (14N–14P–14K) was applied to each pot. The environmental growth chambers were set at 23 °C during the day and 18 °C at night, 60% relative humidity, with a 12:12-h photoperiod provided by fluorescent and incandescent lamps (ratio of irradiance between fluorescent and incandescent lamps = 4:1).

Adult greenhouse whiteflies were collected from commercial strawberry fields in southern California (Oxnard, Ventura County) in 2004 and were immediately used for bioassay experiments. Immature greenhouse whiteflies used in the experiment were developed from eggs laid by the field-collected whitefly adults. Spiromesifen (Oberon 2SC) was obtained from Bayer CropScience, Research Triangle Park, N.C. The insecticide was diluted in deionized water. Nonionic wetter/spreader (Kinetic-1, from Bayer

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CropScience) was added to the insecticide solution at a concentration of 0.1% (v/v).

Bioassay. Leaflets of strawberry plants were sprayed until runoff, ensuring complete coverage of both upper and lower surfaces with a specific amount of the insecticide dissolved in deionized water containing Kinetic-1. Control plants were sprayed with kinetic water solution only. Three to five plants were used for each treatment rate and the control. After the leaf surface was dried, 30 greenhouse whitefly adults were clip-caged on the lower side of a leaflet of the most recently fully expanded trifoliolate (cage size 4 cm² in diameter and 2 cm in depth). Adults were aspirated into pipette tips and gently released into the cages. Adult mortality was determined at 72 h after initial exposure.

For egg and immature greenhouse whitefly bioassay, 40 adults were clip-caged on the lower side of a leaflet of the most recently fully expanded trifoliolate. After an oviposition period of 24 h, the adults were removed. The infested plants were sprayed as described previously with the insecticide solution when eggs (1 d old), first instar (10 d old), second instar (14 d old), and third instar (24 d old) nymphs were present. Egg mortality was determined at 11 d posttreatment, whereas nymph mortality was determined at 14 d post-treatment when they failed to develop into their next stages (instars).

Field experiment

Experimental plots. The strawberry bare-root seedlings (cv. Camarosa) were planted in the Fall of 2003 on four-row beds in a commercial strawberry field in southern California (Bonsall, San Diego County). Each bed was 1.3-m wide and 50-m long. The test was arranged in a randomized complete block design with five replicates. Plot size was 3-m long × 1.3-m wide with a 1-m buffering area between the plots. There were ≈40 plants in each plot.

Insecticide concentrations and applications. Efficacy of spiromesifen against the greenhouse whitefly was evaluated using applications of buprofezin and pyriproxyfen as comparisons. Buprofezin (Applaud 70 WP) was obtained from AgrEvo USA Company, Pikeville, N.C., whereas pyriproxyfen (Esteem 0.86 EC) was obtained from Valent USA Corporation, Walnut Creek, Calif.

Insecticides applied at the label-recommended concentrations were as follows: spiromesifen at 283.8 g·ha⁻¹ a.i., buprofezin at 388.8 g·ha⁻¹ a.i., and pyriproxyfen at 60.0 g·ha⁻¹ a.i. All the insecticides were applied on 29 Mar. 2004 at a volume of 946 L of water per hectare with an ECHO air-assisted sprayer. Control plots were left untreated.

Whitefly sampling methods. Sampling of whitefly adults, immatures, and eggs was initiated 1 week after application of all the insecticides on a weekly basis and ended in mid-May. The 10 youngest and fully expanded middle leaflets of trifoliates, each from a randomly selected plant in each of the plots, and 10 older leaflets from the same

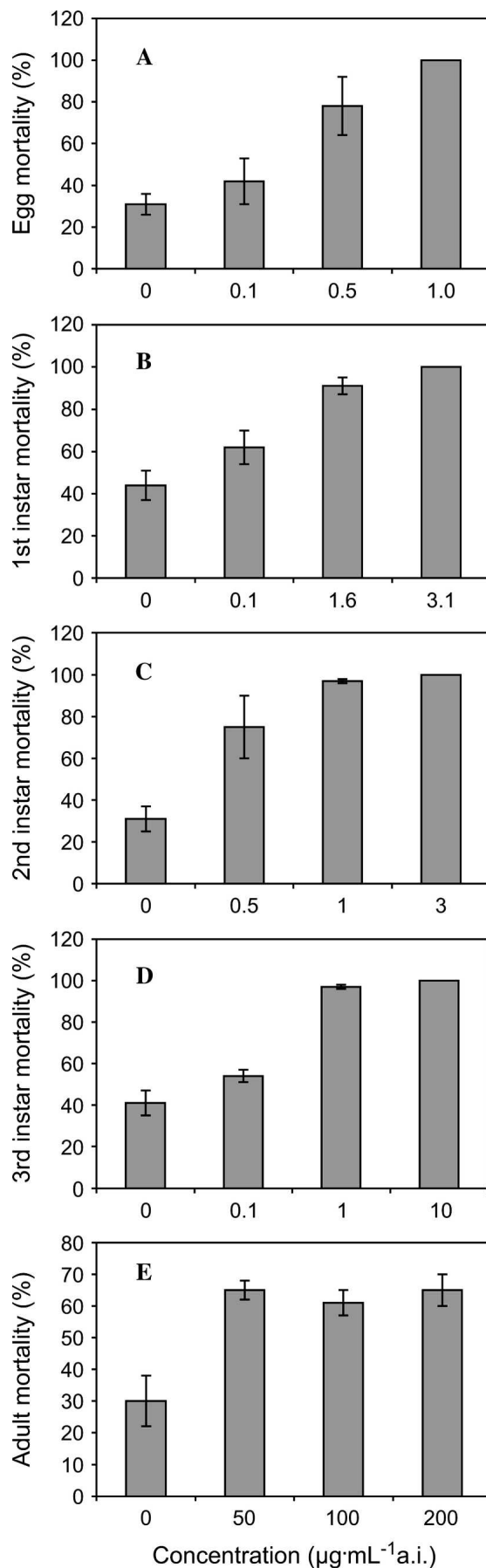


Fig. 1. Effect of spiromesifen treatment on (A) egg hatching rate and mortality of (B) first instar, (C) second instar, (D) third instar, and (E) adult greenhouse whiteflies on strawberry. Error bars represent standard errors.

plants in each plot were excised. These leaflets were placed into plastic zip-lock bags and transported in a cooler to the laboratory to count eggs (on young leaflets) and immatures (on older leaflets) using a stereo dissecting microscope. Adult whiteflies were collected with an engine-powered vacuum (Allen-Vac) (Osborne and Allen, 1999; Bi et al., 2001) over the top of one of the center two rows, either the second or third row (alternated between the center rows in different sampling dates) in each plot. The collected samples were transported to the laboratory and the numbers of adults were counted under a microscope. Numbers of other insect pests in the samples were negligible and not counted.

Statistical analyses. Least significant difference test in one-way randomized complete block design of analysis of variance in SAS (SAS Institute, 2001) was used in the field experiment to analyze the data and separate the means for samples from each sampling date. Before the analysis of variance, numbers of whitefly adults, immatures, and eggs were transformed using the formula $\log(y + 1)$ to normalize the data.

Results

Laboratory experiment. Egg stage of the greenhouse whitefly was very susceptible to spiromesifen (Fig. 1A). Egg hatching was inhibited nearly 80% at a spiromesifen concentration of $0.5 \mu\text{g}\cdot\text{mL}^{-1}$ a.i., whereas complete inhibition of egg hatching occurred at a concentration of $1.0 \mu\text{g}\cdot\text{mL}^{-1}$ a.i., a 300-fold lower than the label recommended rate ($300 \mu\text{g}\cdot\text{mL}^{-1}$ a.i.).

Spiromesifen was highly toxic to immature greenhouse whiteflies (Fig. 1B–D). At a concentration of $1.6 \mu\text{g}\cdot\text{mL}^{-1}$ a.i., spiromesifen caused 91% mortality of the first instar nymphs; and at a concentration of $3.1 \mu\text{g}\cdot\text{mL}^{-1}$ a.i., near 100-fold lower than the label recommended rate, it killed 100% of the nymphs (Fig. 1B). At a concentration of $3.0 \mu\text{g}\cdot\text{mL}^{-1}$ a.i. (100-fold lower than the label recommended rate), this compound killed the second instars completely (Fig. 1C); and at a concentration of $10.0 \mu\text{g}\cdot\text{mL}^{-1}$ a.i. (30-fold lower than the label recommended rate), it killed 100% of the third instars (Fig. 1D).

Spiromesifen was moderately toxic to adult greenhouse whiteflies (Fig. 1E). At concentrations of 50.0, 100.0, and $200.0 \mu\text{g}\cdot\text{mL}^{-1}$ a.i., it killed 65%, 61%, and 65% of the adults, respectively (Fig. 1E).

Field experiment. Application of spiromesifen decreased egg numbers by 55% to 73% ($P < 0.05$) from 2 (12 Apr.) to 5 weeks (3 May) posttreatment, compared with the untreated control, after which the egg numbers were similar ($P > 0.05$) in the treated and the control plots (Fig. 2A). Spiromesifen treatment reduced egg numbers by 61% to 80% ($P < 0.05$) from 2 (12 Apr.) to 3 weeks (19 Apr.) posttreatment in comparison with the pyriproxyfen treatment, after which the numbers were similar ($P > 0.05$) between the two treatments (Fig. 2A). Compared with the buprofezin treatment, spiromesifen appli-

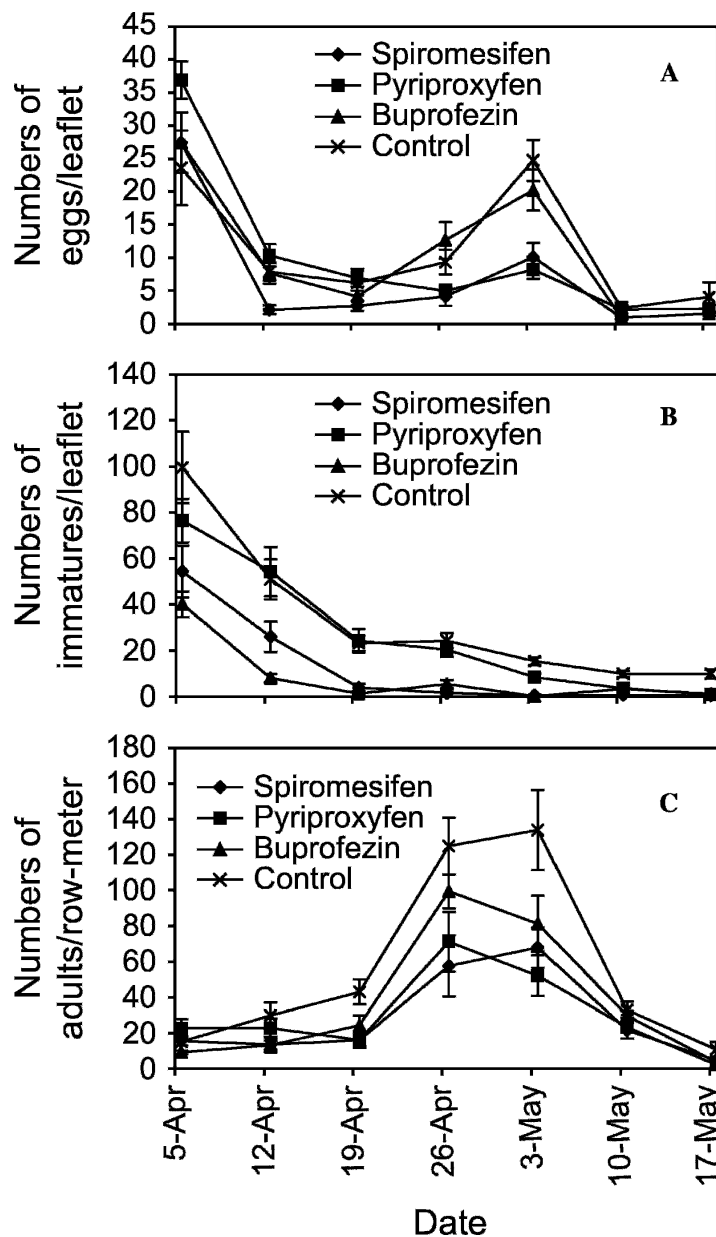


Fig. 2. Efficacy of spiromesifen, pyriproxyfen and buprofezin treatments on numbers of (A) eggs, (B) immatures, and (C) adults of greenhouse whitefly on strawberry. Error bars represent standard errors.

cation lowered the egg numbers by 34% to 73% ($P < 0.05$) from 2 (12 Apr.) to 5 weeks posttreatment (Fig. 2A).

When compared with the untreated control, spiromesifen treatment depressed the numbers of immatures by 45% and 49% ($P < 0.05$), respectively, at 1 (5 Apr.) and 2 weeks (12 Apr.) posttreatment, whereas the numbers were decreased by 79% to 95% ($P < 0.05$) from 3 (19 Apr.) to 7 weeks (17 May) posttreatment (Fig. 2B). In comparison with pyriproxyfen treatment, spiromesifen application reduced the immature numbers by 29% at 1 week posttreatment and by 52% to 92% ($P < 0.05$) from 2 to 6 weeks posttreatment (Fig. 2B). Compared with buprofezin treatment, the effect of spiromesifen on reduction of immature numbers was similar ($P > 0.05$) on all the sampling dates except 12 Apr.,

when the immature numbers were 3.2-fold greater ($P < 0.05$), and 10 May, when the numbers were 79% fewer ($P < 0.05$) (Fig. 2B).

Adult whitefly numbers started to be significantly decreased ($P < 0.05$) from 2 weeks posttreatment (12 Apr.) and the efficacy lasted for another 3 weeks (until 3 May) in spiromesifen treatment compared with the untreated control (Fig. 2C). The decrease in adult numbers ranged from 49% to 62%. The adult numbers in spiromesifen and pyriproxyfen treatments were similar ($P > 0.05$) on all sampling dates. Differences in adult numbers between spiromesifen and buprofezin treatments were also not significant ($P > 0.05$) on all sampling dates except 26 Apr., when the number was 42% greater ($P < 0.05$) in the buprofezin treatment than in the spiromesifen treatment (Fig. 2C).

Discussion

Our laboratory test revealed that spiromesifen was very effective in inhibiting the whitefly egg hatching, highly toxic to the immature whiteflies, and moderately effective in killing the adult whiteflies on strawberry (Fig. 1A–E). Nauen et al. (2002) reported that spiromesifen was especially active against the greenhouse whitefly, particularly in the juvenile stages on cotton. Liu (2004) showed spiromesifen was highly toxic to nymphs and slightly toxic to adults of silver-leaf whitefly (*B. tabaci* Gennadius) on both melons and collards (*Brassica oleracea* L.). However, Liu's results indicated that spiromesifen was nontoxic to *B. tabaci* eggs (Liu, 2004). The difference in inhibition of egg hatching of the greenhouse whitefly and the silver-leaf whitefly may be the result of the differences in whitefly species or host plant species.

Pyriproxyfen is an insect growth regulator affecting the hormonal balance in insects, whereas buprofezin is another insect growth regulator inhibiting chitin synthesis in insects (De Cock et al., 1990; Ishaaya and Horowitz, 1998). We previously conducted both greenhouse and field experiments to evaluate pyriproxyfen and buprofezin against the greenhouse whitefly on strawberry in southern California (Bi et al., 2002a, 2002b). In the greenhouse experiment, we showed that pyriproxyfen was excellent in inhibition of egg hatching of the whiteflies, whereas buprofezin is highly effective against growth and development of the immatures (Bi et al., 2002a, 2002b). In the field experiment, we demonstrated that both pyriproxyfen and buprofezin significantly suppressed the whitefly populations on both fall- and summer-planted strawberry (Bi et al., 2002a, 2002b). As a result of the excellence in controlling their target insects, low toxicity to mammals, and relative safety to most parasitoids, buprofezin and pyriproxyfen are considered as important components of integrated greenhouse whitefly management programs. In this study, our laboratory experiment showed that spiromesifen was highly effective against both eggs and the immatures of the greenhouse whitefly and moderately effective against the adults (Fig. 1A–E). Our field experiment clearly indicated that spiromesifen was superior to pyriproxyfen in reducing the whitefly egg and immature numbers (Fig. 2A, B). Spiromesifen was also superior to buprofezin in decreasing the whitefly egg numbers (Fig. 2A). The effect of spiromesifen on reduction of the immature numbers was similar to that of buprofezin (Fig. 2B).

It was reported that spiromesifen is safe on beneficial organisms and has a favorable environmental profile (Nauen et al., 2002). Spiromesifen is also extremely effective against pyriproxyfen-resistant whiteflies and no crossresistance to any important insecticide and acaricide was found (Nauen et al., 2002). Together with results in this study, we conclude that spiromesifen can be a new component of the integrated greenhouse whitefly management programs on strawberry and can be a new valuable tool in the whitefly resistance management programs.

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