Effect of Milbemectin on the Sweetpotato Whitefly, *Bemisia tabaci*

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Milbemectin has a chemical structure close to the group of avermectins, which are derived from *Streptomyces avermitilis*, and is considered primarily an efficient miticide. Effects of milbemectin on the sweetpotato whitefly, *Bemisia tabaci* Gennadius, were investigated under laboratory and field conditions. In bioassays conducted under controlled chamber conditions, the compound affected 1st instars of *B. tabaci*, resulting in a LC_{90} of 0.06 mg a.i. l^{-1}. Later stage larvae were much less affected. Milbemectin is highly photodegradable in sunlight. In laboratory assays, when treated cotton seedlings were subjected to 3 h of sunlight before being exposed to *B. tabaci* adults, no mortality of the whiteflies was observed. Milbemectin at a concentration of 2 mg a.i. l^{-1} applied in combination with 0.2% 'Ultra Fine' mineral oil showed a residual activity of 67% adult mortality 10 days after application, whereas milbemectin alone had no appreciable activity. The effect of milbemectin on whitefly populations in a cotton field was compared with that of cypermethrin and of untreated control. Although milbemectin was not applied with mineral oil, it was more effective than cypermethrin in controlling the whitefly populations. This insecticide/miticide seems not to affect appreciably natural enemies of *B. tabaci*. Milbemectin may be considered a compound with the potential for controlling *B. tabaci* populations. Mineral oils enhanced the potency of milbemectin on both whitefly larvae and adults.

KEY WORDS: *Bemisia tabaci*; milbemectin; mineral oil; avermectins; larval mortality; parasitoids.

INTRODUCTION

Milbemectin exhibits appreciable miticidal activity, affecting a wide range of important mite pests such as the twospotted spider mite (*Tetranychus urticae* Koch), the carmine spider mite [*Tetranychus cinnabarinus* (Boisd.)] and the citrus red mite (*Panonychus citri* McGregor) (29). The product is a mixture of milbemycin A3 and milbemycin A4, both of which are metabolites of *Streptomyces hygroscopicus* subsp. *aureolacrimosus* (2). The closely related chemical structures of milbemycins and avermectins (1,12,23) led to the assumption that milbemectin may have insecticidal activities against important pests, as has been found with the avermectins abamectin and emamectin (11,16,24,27). Deecher *et al.* (8) describe insecticidal effects of milbemycin D on the gypsy moth, *Lymantria dispar* (L.).

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Our findings have indicated that milbemectin is a compound of potential use against the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), a cosmopolitan pest of many field and garden crops (3). A large population of whiteflies may reduce crop yield. In addition, excretion of whitefly honeydew on the plant, followed by development of black sooty mold fungi, lowers crop quality and value. *B. tabaci* is a vector of viral diseases and is considered to be a limiting factor for the production of some vegetables (especially tomatoes) and ornamentals (4,5,28).

Intensive whitefly research has been conducted to evaluate insecticides with novel modes of action and with minimum harm to man and natural enemies (17,18), such as the chitin synthesis inhibitor buprofezin (22), the thiourea derivative diafenthiuron (21) and the juvenoid compound pyriproxyfen (20). After successive use of pyriproxyfen and buprofezin, a relatively high resistance was recorded in some rose and tomato greenhouses (6,15). Urgent research is required to evaluate insect control agents with different modes of action to be used in rotation for controlling whiteflies in cotton, vegetables and ornamentals.

In this paper, data on the effectiveness of milbemectin against *B. tabaci* under laboratory and field conditions and its impacts on natural enemies are analyzed.

MATERIALS AND METHODS

Chemicals

Milbemectin (CM-006; Sankyo Co. Ltd., Japan, obtained from Agan Chemicals Ltd., Ashdod, Israel), 1% suspension concentrate (SC), was used in laboratory and field experiments. The formulation was diluted with deionized water to the desired concentrations. ‘Ultra Fine’ mineral (paraffinic) oil (98.8%, Sunoil, Philadelphia, PA, USA) was obtained from S. Riesel Chemicals, Ramat Gan, Israel. In the field trials milbemectin was compared with Cymbush (10% a.i. of cypermethrin; emulsifiable concentrate (EC); Zeneca Agrochemicals, obtained from Makhteshim Chemicals, Israel).

Rearing and bioassay

Bemisia tabaci were reared on cotton seedlings under standard laboratory conditions of 25±1°C, 65±5% r.h. and a photoperiod of 14:10 (L:D) (20). Effects of milbemectin on eggs and larval stages of *B. tabaci* were determined by dipping cotton seedlings infested with eggs, 1st or 3rd instar larvae in various concentrations of the compound or in water as control. Cotton seedlings (20–25 cm high) infested with 0–1-day-old eggs, 1st or 3rd instar larvae were subsequently treated with various concentrations of milbemectin. Egg and larval mortality were determined 7 and 10 days, respectively, after application of the compound. In other assays, cotton seedlings were dipped in various concentrations of milbemectin. After 2 h of air-drying, plants were exposed to *B. tabaci* females confined in leaf cages (19,22), 15 individuals per cage, for 48 h under controlled conditions as mentioned above. The effects on adult mortality, fecundity, egg viability, cumulative larval mortality (expressed in suppression of pupation) and adult formation were determined. Residual activity of milbemectin alone, or in combination with 0.2% ‘Ultra Fine’ mineral oil, was estimated by exposing *B. tabaci* females to treated cotton seedlings for 48 h (as mentioned above) at various periods after application (3 h and 3, 10, 15 days). The treated seedlings were subjected to sunlight for 3 h before their exposure to whiteflies.

Cotton seedlings dipped in sublethal concentrations of milbemectin (0.06, 0.18 and 0.54 mg a.i. l^{-1}) were air-dried for 24 h and then exposed for 48 h to whitefly females.

Effects of milbemectin on adult mortality, fecundity, egg hatch, cumulative larval mortality (expressed in suppression of pupation) and adult emergence were determined.

Field trials

In a field trial, the effect of milbemectin on *B. tabaci* and natural enemy populations was investigated. The study was conducted during the 1997 cotton-growing season in experimental plots of cv. 'Acala', *Gossypium hirsutum* L., at Bet Dagan (central coastal plain of Israel). The field trial was laid out in a randomized block comprising four replicates per treatment; the size of each replicate was 8×4 m. The effect of milbemectin on the whitefly population was compared with that of cypermethrin and of untreated control. Plots were sprayed using a backpack-motor sprayer with 200 l of spray solution per ha. Milbemectin was sprayed three times and cypermethrin four times during the cotton season (see Fig. 1). Levels of infestation of *B. tabaci* were measured weekly by counting pupae on the most heavily infested leaf ('maximal leaf') of ten randomly sampled cotton plants per plot (14). Rate of parasitism by *Eretmocerus* sp. and *Encarsia* sp. was determined by counting parasitized pupae on the collected leaves.

Statistical analysis

Probit regression using POLO-PC analysis (25) was employed for determining the slope and LC values. All results were subjected to analysis of variance (ANOVA) and means were separated by the SNK test. Data presented as percentages underwent angular transformation prior to statistical analysis.

RESULTS AND DISCUSSION

Egg viability and larval mortality

Egg viability was affected only slightly by application of relatively high concentrations of milbemectin (Table 1). Concentrations of 5 and 25 mg a.i. l^{-1} were necessary to obtain a suppression in egg hatch of approximately 30%. However, newly hatched larvae died close to their eggshells, even if the eggs were exposed to a concentration as low as 1 mg a.i. l^{-1} milbemectin.

TABLE 1. Effect of milbemectin on egg viability and newly hatched larvae of *Bemisia tabaci*

Milbemectin Conc. (mg a.i. l^{-1})	No. of eggs	Egg hatch (% ± SEM)	Mortality of 1st instars (%)
0	695	89±4a	0
1	653	81±10ab	100
5	741	60±5b	100
25	640	66±5b	100

Cotton seedlings infested with eggs of *B. tabaci* were treated with various concentrations of milbemectin. Effect on egg viability expressed by egg hatch and on mortality of newly hatched larvae was determined. Data are averages ± SEM of five replicates of 96–177 *B. tabaci* eggs (0–24 h old) each. Means followed by the same letter do not differ significantly at *P*=0.05 (SNK multiple range test). Angular transformation of egg hatch was done prior to statistical analysis.

Bioassays done with 1st instars confirmed our observation of high susceptibility of young larvae to milbemectin. At a concentration of 0.1 mg a.i. l^{-1}, the mortality rate reached 100% (LC_{90} value was 0.06 mg a.i. l^{-1}) (Table 2). Hence, milbemectin showed its ability to control effectively young *B. tabaci* larvae. Milbemectin affected *B. tabaci* 3rd instar larvae to a much lesser extent, resulting in a LC_{90} value of approximately 40 mg a.i. l^{-1} (Table 2).

TABLE 2. Slopes and LC-values (mg a.i. l^{-1}) of milbemectin on 1st and 3rd instars of *Bemisia tabaci*

Toxicity data	1st instar larvae	3rd instar larvae
Slope ±SEM	2.42±0.14	0.70±0.04
LC_{50} (95% C.I.)	0.02 (0.01-0.03)	0.59 (0.31-1.22)
LC_{90} (95% C.I.)	0.06 (0.04-0.08)	40.12 (10.50-672)

Data based on 5–13 replicates of 20–164 1st instars each, and 5–9 replicates of 29–142 3rd instars each. Six concentrations were used for determining mortality curves and LC-values. Calculations were done by POLO analysis procedure (25).

Horowitz *et al.* (16) studied the effect of abamectin on *B. tabaci* larvae. The potency of abamectin on 1st instars was similar to that found in our study. Dybas (11) found a relatively high potency of abamectin against mites, spider mites, some beetles, and lepidopteran pests and low efficacy against various thrips and aphids. Deecher *et al.* (7) have shown that abamectin was much more potent than milbemycin D on *L. dispar* 3rd instars.

Effects of sublethal dosages on progeny formation

No adult mortality occurred and egg-hatch of *B. tabaci* was not affected by the treatments; some effect on fecundity was observed with the two highest concentrations in this assay (Table 3). Young larvae showed a relatively high susceptibility at a concentration of 0.54 mg a.i. l^{-1}. These larvae died mainly during the first and second instars. Dead larvae were found not only close to their eggshells, and hence it could be assumed that the larvae were affected in part by sucking the compound *via* the phloem. From our observations, 3rd instars were not affected and most of them were able to pupate. Very little pupal mortality was observed, resulting in almost no reduction in adult formation.

Residual activity and improving penetration

Like avermectins, the milbemycins are photodegradable in sunlight (26). In order to improve residual activity in the field, the penetration of the compound into a cotton leaf should be increased. Horowitz *et al.* (16) showed that addition of 'Ultra Fine' mineral oil increased residual potency of abamectin. They assumed that mineral oil enhances the translaminar activity of the insecticide. Hence, in the present bioassays (Table 4), cotton seedlings treated with milbemectin alone or in combination with 'Ultra Fine' oil were exposed to 3 h of sunlight. Milbemectin applied alone at a concentration of 10 mg a.i. l^{-1} brought about no mortality of *B. tabaci* adults. However, application of 'Ultra Fine' alone kept the mortality at 75% until the third day after treatment. A combined treatment of milbemectin and mineral oil resulted at day 3 after treatment in adult mortality of over 90%, and of approximately 60% and 30% on days 10 and 15, respectively. There was no significant difference between the two tested concentrations of milbemectin (2 and 10 mg a.i. l^{-1}) when applied with 'Ultra Fine' (Table 4). The residual activity observed by addition of 'Ultra Fine' in the present study was somewhat weaker than that found in assays with abamectin (16).

TABLE 3. Effect of sublethal dosages of milbemectin on *Bemisia tabaci* adult mortality, fecundity and progeny formation

Milbemectin Conc. (mg a.i. l^{-1})	Adult mortality (% ±SEM)	No. of eggs/female (±SEM)	Egg hatch (% ±SEM)	Pupation (% ±SEM)	Adult emergence (% ±SEM)
0	0±0a	6.3±0a	99±1a	98±2a	82±4a
0.06	9.9±4a	6.0±1ab	99±1a	90±4ab	69±5a
0.18	4.3±3a	4.5±0b	97±2a	75±8b	67±8a
0.54	6.3±2a	4.0±0b	96±1a	22±7c	19±6b

Cotton seedlings were treated with various concentrations of milbemectin and with deionized water as control. After 2 h of air drying, the treated seedlings were exposed to whiteflies for 28 h. Adult mortality along wtih fecundity (eggs/female), egg hatch, pupation and emergence were determined. Data are averages ± SEM of five replicates of 15 adult females each. Within columns, means followed by the same letter do not differ significantly at P=0.05 (SNK multiple range test). Data presented in percent underwent angular transformation prior to statistical analysis.

TABLE 4. Residual activity of milbemectin applied alone or in combination with 'Ultra Fine' mineral oil on *Bemisia tabaci* adults

Milbemectin Conc. (mg a.i. l^{-1})	'Ultra Fine' (%)	Adult mortality (% ±SEM) at various days after treatment			
		0 (3 h)	3	10	15
–	–	4±6a	1±1a	7±4a	7±2a
10	–	6±2a	29±10b	7±3a	9±3a
–	0.2	96±2b	75±7c	12±5a	21±2a
2	0.2	93±4b	96±2d	67±10b	32±11a
10	0.2	94±5b	97±2d	59±10b	31±18a

Cotton seedlings were treated with milbemectin applied alone or together with 'Ultra Fine' mineral oil. The treated seedlings were kept under sunlit conditions for 3 h and exposed to whiteflies at various days after treatment for 48 h; adult mortality was then determined. Data are averages ±SEM of five replicates of 15 adult females each. Within columns, means followed by the same letter do not differ significantly at P=0.05 (SNK multiple range test). Angular transformation was done prior to statistical analysis.

Effects of milbemectin on B. tabaci *and its parasitoids under field conditions*

In the cotton field trials the potency of milbemectin in controlling whitefly populations was compared with that of cypermethrin and of untreated control. Judging from the number of pupae per 'maximal leaf', milbemectin gave the best control, with a significant difference from the cypermethrin treatment (Fig. 1). The relatively reduced whitefly population in the control and in the milbemectin treatment as compared with cypermethrin results probably from a high level of natural enemies present in the plots.

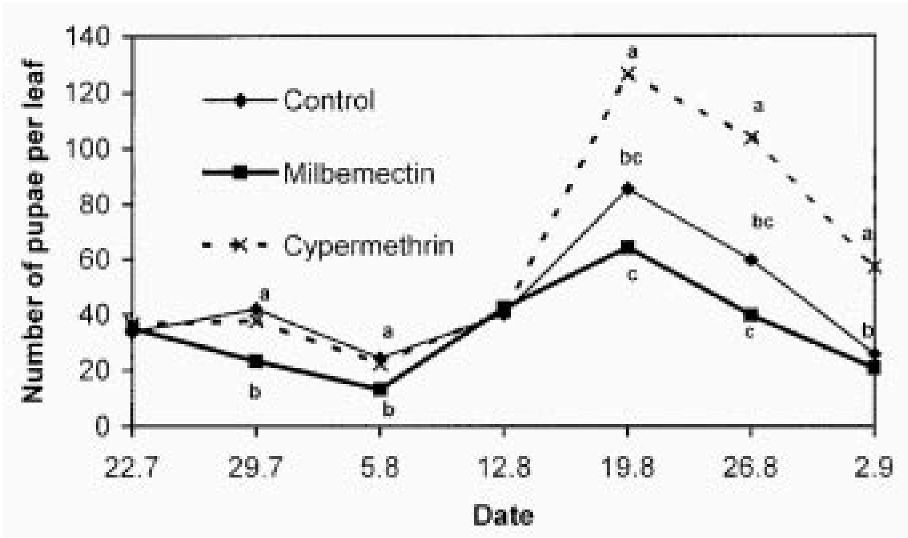

Fig. 1. Effects of milbemectin and cypermethrin on *Bemisia tabaci* field populations (expressed in number of pupae per maximal leaf), Bet Dagan, 1997. Different letters within the same sampling date differ significantly at P=0.05. Milbemectin was sprayed three times at a concentration of 50 mg a.i. l^{-1} (on July 23, August 3 and August 19); cypermethrin was sprayed four times at a concentration of 750 mg a.i. l^{-1} (on July 23, August 3 , August 13 and August 19).

Although milbemectin is photodegradable, it could affect the *B. tabaci* population probably through its penetration into the cotton leaf (translaminar activity). Further assays are required using milbemectin in combination with mineral oil.

The effect of the treatments on the parasitoid wasps *Eretmocerus* sp. and *Encarsia* sp. was checked by determining parasitism of pupae on the collected leaves. The first substantial parasitism of pupae was observed on August 19 and increased in later weeks, but statistical analysis showed that there were no significant differences between treatments (Fig. 2). None of the compounds used had a measurable effect on parasitism; Gerling (13) obtained similar results in cotton fields. In his investigations, the population of *B. tabaci* rose in the middle of August and declined in both treated and untreated plots in the same manner. Parasitism increased later with a higher percentage in treated plots. He concluded that both whiteflies and parasitoids were protected during this period to some extent by the closed canopy of the cotton field. The lack of effect on parasitoids in the case of cypermethrin is possibly due to a general lack of effect, as a result of resistance development in both the treated pest (9) and its parasitoids. The relatively high whitefly infestation in the cypermethrin plots may result rather from the negative impact on the predators in the field (10). In the later stages, when wasps lay their eggs into or under the larval surface, the compounds are much less effective. In this way, the parasitoids are protected by their hosts. The slight increase in the percentage of parasitism observed in the three plots at the end of the season may result from lower numbers of pupae present in the field. The whitefly populations were reduced in treated plots due to the effect on young larvae, whereas the parasitoids present in 3rd instars and pupae were not affected.

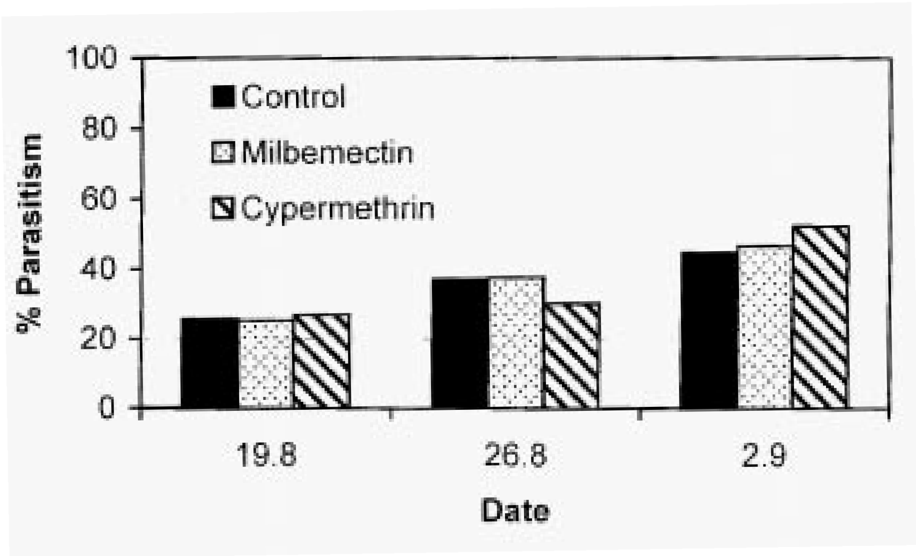

Fig. 2. Impact of milbemectin and cypermethrin on *Bemisia tabaci*–parasitoid field populations, Bet Dagan, 1997.

In this study we found that milbemectin has a moderate effect on whitefly populations; when it is sprayed alone, only young larvae are affected. Combining the compound with 'Ultra Fine' mineral oil can enhance the effects of milbemectin on whitefly larvae and adults. In addition, milbemectin may prove to be more efficient under greenhouse conditions than in the field, due to lower photodegradation. Additional investigations are required to determine the effect of this combination on adults and developing stages of *B. tabaci* and its parasitoids.

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