

## Biological Control of the Western Flower Thrips *Frankliniella occidentalis* in Cucumber Using the Entomopathogenic Fungus *Metarhizium anisopliae*

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Biological control of the western flower thrips (WFT) *Frankliniella occidentalis*, using the entomopathogenic *Metarhizium anisopliae*-7 (*M. a-7*) strain was studied in three consecutive seasons under greenhouse conditions. Cucumber plants infested with WFT were sprayed with spore suspension of the fungus *M. a-7* ( $0.5 \text{ g m}^{-2}$ ), or the soil was treated with dry powder of the fungus ( $0.5 \text{ g m}^{-2}$ ); the control was without fungus application. In the 1997 spring experiment, when the cucumber plants were initially infested with only three or four insects per leaf, the spore suspension spray caused a significant reduction in growth of the thrips population compared with the other treatments and the control. However, in the 1997 summer experiment, when the plants were initially heavily infested with WFT (10–15 insects per leaf), the spray treatment caused only a modest reduction in WFT population growth, and only after 4 weeks of treatment was the reduction significant. In the 1999 experiment, with a low initial WFT population of three or four insects per leaf, the spray treatment was effective in reducing the population growth to a lower level than in the other treatments or control. The *M. a-7* strain was found to be effective in reducing the population growth of WFT under greenhouse conditions, particularly when the initial thrips population was low to moderate.

KEY WORDS: *Metarhizium anisopliae*; *Frankliniella occidentalis*; biocontrol; entomopathogenic fungi; pathogenicity; bioinsecticide.

### INTRODUCTION

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is highly destructive to various crops of economic importance including vegetables, ornamentals, field crops and fruits (3,16,17,19). Chemical control of WFT is often difficult for a few reasons: the insect has developed resistance to many insecticides because of its life cycle, which includes two pupal stages (in soil); and due to the cryptic behavior of adults and nymphs, which are generally found in flowers or in growing terminals (10,14,15). WFT is a vector for the tomato spotted wilt virus (TSWV), and is considered to be a major pest in Israel and elsewhere (12).

Bioassays have revealed that several isolates of the fungus *Metarhizium anisopliae* are highly virulent toward WFT adults, and some isolates germinate rapidly on the surface of nymphs, pupae and adults when tested in field experiments (18). *M. anisopliae* is a

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potential biological control agent which has been isolated from soil and diseased insects (9,20), and has been used to control pest insects for many years (7,8,11). Some microbial products based on *M. anisopliae* have been registered in the USA and Australia (4,13).

The WFT is a relatively new pest in Israel (1) and is considered a major pest in greenhouses and in field crops; it develops well during the warm spring and hot summer in Israel. An indigenous strain of *M. anisopliae* (*M. a-7*) was selected as a biological agent with high pathogenicity toward WFT; it was found to be a temperature-tolerant isolate in early studies (2). The objective of the present study was to determine the efficacy of the strain *M. a-7* in controlling WFT on cucumber plants cultivated in greenhouses.

## MATERIALS AND METHODS

**Inoculum preparation for greenhouse experiments** Strain *M. a-7* was cultivated at 25°C for 3 weeks on water-soaked wheat grains (swelling grains) which had been sterilized twice. The fungus and the grains were dried at 34–35°C for 2 days, after which the inoculum was ground to powder in a few minutes using a blender (Braun, Kronerj, Germany). To determine spore number per gram of dry formulation, a 0.01 g sample was mixed with 10 ml of water containing 0.005% Triton X-100 (v:v) using a vortex for 10 sec. The number of spores was determined with a hemocytometer. For the field tests, the dry formulation containing  $2$  to  $4 \times 10^9$  spores  $g^{-1}$  was mixed with water with 0.005% Triton X-100 (1 g spores in 100 ml solution) and the spore suspension was filtered through several layers of cheesecloth to remove grain mats and then was diluted to  $\sim 5 \times 10^7$  spores  $ml^{-1}$ . The number of colony forming units was determined by plating samples of spore suspension at an appropriate dilution on Czapek agar medium prior to inoculation.

**1997 Spring Experiment** The experiment was conducted in the Galilee region on cucumber plants (6 weeks old, cv. BA) cultivated in heavy soil inside a greenhouse with drip irrigation. The plants were naturally infested with three or four WFT per leaf (considered to be a medium level of infestation). Four treatments were tested: (i) control without spore treatment; (ii) plants sprayed with the spore suspension ( $5 \times 10^7$  spores  $ml^{-1}$ ) at a rate of 0.5 g dry formulation per  $m^2$ , using a hand sprayer to avoid drift onto control plants; (iii) soil treatment with ground inoculum at a rate of 0.5 g dry formulation per  $m^2$ , mixed into soil by hand; and (iv) a second control, consisting of plants sprayed with water only.

The experiment was a randomized block design, with two 10- $m^2$  blocks per treatment and three traps per block (six traps per treatment). The insect populations in the different treatments were monitored once a week by trapping the WFT adults using blue sticky traps. Each trap consisted of a 9-cm-diam petri dish containing a layer of the sticky material Rimifoot (80% polyisobutane and 20% formulation compounds; Jewnin-Joffe Industry Ltd., Israel) which were hung 70 cm above the ground. The insect population was determined 1 week before the first application. A second application of each treatment was done 2 weeks later. The plants were sprayed routinely to control fungal diseases only (the routine fungicide applications were withheld for 1 week after the fungal spore treatment). No chemicals were used at the experiment site to control thrips. The traps were monitored once a week for 5 weeks.

**1997 Summer Experiment** This experiment was conducted in a different greenhouse in the same region as the 1997 spring test, to control WFT in cucumber plants (7 weeks old,

cv. BA) heavily infested with thrips (10 to 15 insects per leaf). The same four treatments were used as in the spring experiment. The experiment was a randomized block design with three 10-m<sup>2</sup> blocks per treatment and three traps per block (nine traps per treatment).

**1999 Spring Experiment** This experiment was conducted in a different greenhouse in the same region as the 1997 experiments, to control WFT in cucumber plants (4 weeks old, cv. IV-40) with a low level of thrips infestation (two or three insects per leaf). The four treatments were the same as in 1997, except that the second application of the inoculum was conducted 1 month after the first application. The experiment was a randomized block design with three 10-m<sup>2</sup> blocks per treatment and three traps per block (nine traps per treatment).

**Greenhouse environment** The temperature inside the greenhouse was monitored during the spring experiments. During the night hours, the temperature was between 24 ° and 27°C (11 h); at midday the highest temperatures recorded ranged between 38 ° and 42°C (for 2 to 4 h); during the rest of the day the temperature was between 27 ° and 37°C (9 to 11 h). The relative humidity ranged between 70% and 80% during the night and between 40% and 60% during the day.

**Statistical analysis** The data were analyzed using Duncan's multiple range test. The data are presented as means ± standard errors.

## RESULTS AND DISCUSSION

The effect of the highly virulent and temperature-resistant *M. a-7* strain of *M. anisopliae* (2) on the growth in the thrips population size on cucumber plants was tested during three consecutive seasons. The results of the control treatment with water only were the same as the control without water (data not shown); therefore, only one control treatment is recorded in the figures – that without water. The mean initial population of WFT in the 1997 spring experiment was 5±1 insects/trap/week (Fig. 1). The thrips population in control and in soil treatments was similar throughout the experiment (during 6 weeks of plant cultivation). The spray treatment with spores showed significantly lower ( $P<0.05$ ) thrips population throughout the experiment compared with the two other treatments (weeks 2 through 5, Fig. 1). The thrips population in the spray treatment 4 weeks after the first application was 3.6 times less than that in the control, a significant difference ( $P<0.05$ ) (Fig. 1).

In the summer 1997 experiment, the initial population of thrips was relatively high, 70±22 insects/trap/week (Fig. 2). Again, insect populations in the control and in the soil treatment were similar throughout the experiment (4 weeks). Spray treatment did not affect the thrips population significantly until week 3; and only at week 4 did it cause a significant difference in the population growth of WFT compared with the two other treatments ( $P<0.05$ ).

The results of the 1999 experiment showed that spray treatment resulted in a significantly lower ( $P<0.05$ ) population of WFT during weeks 4 through 8 compared with the control (Fig. 3). In week 8, the number of thrips in the soil treatment was similar to that of the spray treatment. This may have been caused by infestation of some of the plants with predator mites, which reduced the thrips population; we did not use any insecticides. In week 8, the thrips population in the control was 2.3-fold greater than in the spray treatment (Fig. 3).

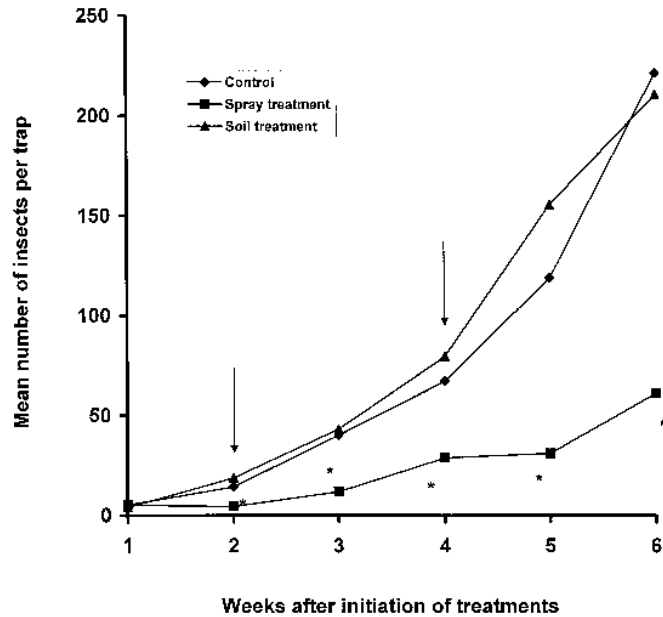


Fig. 1. Effects of three different treatments with *Metahizium anisopliae* strain *M. a-7* on the population of *Frankliniella occidentalis* on greenhouse-cultivated cucumber plants. 1997 spring experiment. Arrows indicate times of the two applications. Each data point represents the mean value of six traps. \*At the same week, data of the spray treatment differ significantly from the two other treatments ( $P < 0.05$ , Duncan's MRT).

Spray treatment with spores of the entomopathogenic strain *M. a-7* to control WFT showed promising results, especially when the thrips population on cucumber was at low to medium levels (Figs. 1 and 3) compared with a high level (Fig. 2). The level of infestation with thrips is apparently an important parameter, in addition to the number of sprays applied during the growth season. We used only two sprays; possibly a third spray would be recommended for high levels of infestation, e.g. three sprays at 2-week intervals.

Some fungi are extremely sensitive to prolonged exposure to high temperatures or to marked fluctuations in temperature (5). The high temperatures prevailing during some of the hours every day in our experiments apparently had negative effects on sporulation and thrips infection, despite the fact that the strain used is relatively resistant to high temperatures (2). We suggest that a special formulation be produced to protect the spores from high temperatures and low r.h., which might lead to even better results in controlling WFT. A good formulation could protect the spores from adverse environmental effects and ensure that when the conditions are favorable for spore germination, the spores will initiate their life cycle.

The only promising biological control results that have been reported for WFT on cucumber under greenhouse conditions were obtained by using predators, e.g. *Dicyphus tamaninii* (6). The 3:10 ratio of *D. tamaninii*: WFT kept the thrips population below economic damage level when the thrips population was relatively high (16 thrips/400

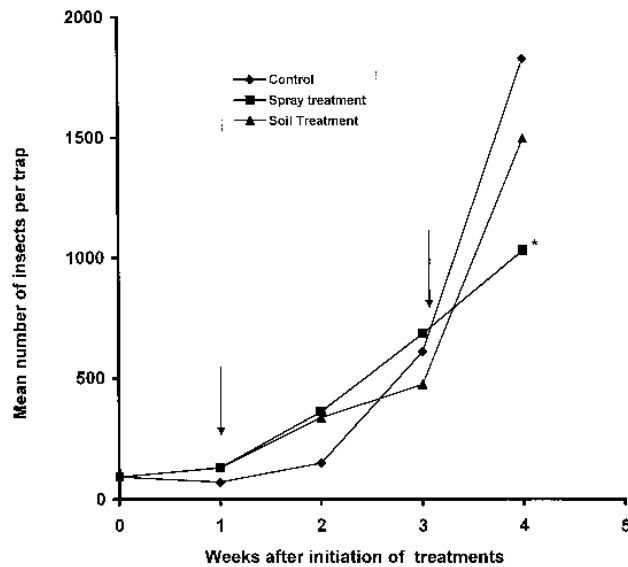


Fig. 2. Effects of three treatments with *Metahizium anisopliae* strain *M. a-7* on the population of *Frankliniella occidentalis* on greenhouse-cultivated cucumber plants. 1997 summer experiment. Arrows indicate times of the two applications. Each data point represents the mean value of nine traps. \*At the same week, data of the spray treatment differ significantly from the two other treatments ( $P < 0.05$ , Duncan's MRT).

cm<sup>2</sup> leaf area). A ratio of 1:10 worked well when the thrips population was low, but not when it was high. Therefore, two sprays of *M. a-7* spores at initial low to medium level of infestation (2–4 thrips/leaf) are good for successful biological control, and a third application is recommended. The three different experiments were conducted in the same area (Sandala, Israel), but the environmental conditions inside the greenhouses were different, which might – in addition to the initial level of thrips infestation – have affected our results.

Strain *M. a-7* is relatively resistant to some fungicides (*i.e.*, difenoconazole and pyrimethanil) at low concentrations (data not shown). It is a local isolate and relatively resistant to high temperatures, but under greenhouse conditions of high temperatures and low relative humidity is not highly efficacious in controlling WFT. Research is currently being conducted to produce appropriate formulations of the effective entomopathogenic isolates for testing under field conditions.

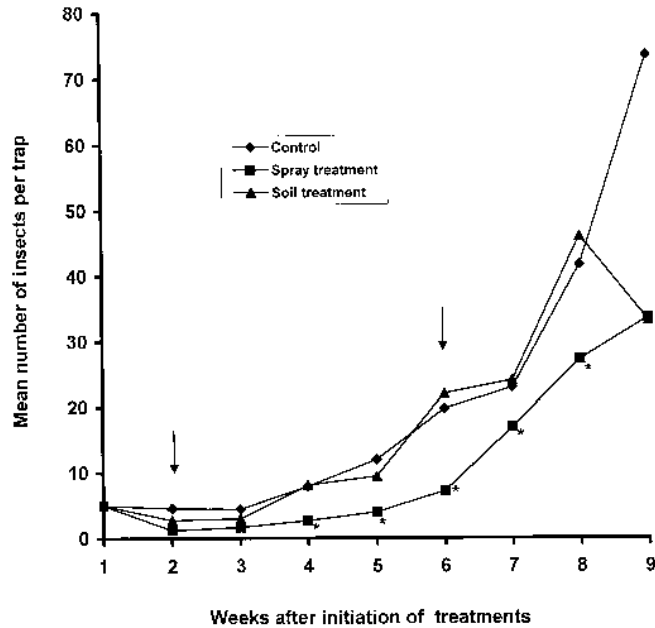


Fig. 3. Effects of three treatments with *Metarhizium anisopliae* strain *M. a-7* on the population of *Frankliniella occidentalis* on greenhouse-cultivated cucumber plants. 1999 spring experiment. Arrows indicate times of the two applications. Each data point represents the mean value of nine traps. \*At the same week, data of the spray treatment differ significantly from the two other treatments ( $P < 0.05$ , Duncan's MRT).

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