Efficacy of Fluazinam in Suppression of *Monosporascus cannonballus*, the Causal Agent of Sudden Wilt of Melons

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ABSTRACT

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Sudden wilt (vine decline) of melon, caused by *Monosporascus cannonballus*, is a worldwide problem in arid and semi-arid regions. Soil disinfestation by fumigation with methyl bromide before planting is a common treatment for disease management but, because methyl bromide is expected to be banned from use within the next 10 years, alternative measures for disease suppression are needed. The efficacy of 29 fungicides against *M. cannonballus* was evaluated in vitro. Among the fungicides tested, fluazinam and kresoxim methyl were the most effective and both totally inhibited the growth of *M. cannonballus* in culture at concentrations of 10 µg a.i./ml. Because fluazinam also was effective in inhibition of *Pythium aphanidermatum*, which also may be involved in sudden wilt syndrome, and kresoxim methyl was not, fluazinam was chosen for further tests. The effective dose of fluazinam for *M. cannonballus* that reduced mycelial growth by 50% was 0.09 µg a.i./ml. Fluazinam efficacy was evaluated in three field experiments conducted in the spring and in the late summer cropping seasons. In two of the experiments, applications of fluazinam resulted in approximately 87% wilt reduction, whereas in the third experiment it was only 32%. The mobility of fluazinam in soil was determined in samples taken from the field. Fungicide mobility in soil was relatively limited; most of the compound was adsorbed to soil particles, resulting in a zone of high concentration that decreased with depth and distance from the application site. Nevertheless, rates measured even at a depth of 25 cm were sufficient to control *M. cannonballus*. This study shows that fluazinam may be used as one component in an integrated approach for suppression of sudden wilt of melons.

Additional keywords: chemical control, *Cucumis melo*, integrated pest management

Sudden wilt of melon (*Cucumis melo*), also known as "melon collapse" and "vine decline," is a worldwide problem (15). The syndrome is particularly important in arid and semi-arid regions such as the 'Arava region in southern Israel. Sudden wilt and the involvement of *Monosporascus cannonballus* in the disease have been reported from the southern United States, Central America, Japan, Tunisia, Saudi Arabia, and Spain (2,7,9,13,14,16-18,27). The disease causes a rapid wilt of plants bearing mature

fruit and may result in a total loss of the marketable yield. Sudden wilt of melons has been known in Israel for two decades (6,12,19-22). In pathogenicity tests performed in the 'Arava in 1995 and 1996, *M. cannonballus* (1) was confirmed to be the most aggressive species involved in the sudden wilt of melons. In these tests, *Pythium aphanidermatum* and *Fusarium solani* also induced wilt symptoms, but to a lesser extent (19).

Fungicides are used frequently to suppress soilborne pathogens (24). Soil disinfestation by fumigation with methyl bromide before planting is, in general, highly effective against sudden wilt of melon and is a common treatment in Israel (26). In fact, due to the severe losses caused by the disease, melon cultivation in the 'Arava region is practically impossible without methyl bromide fumigation prior to planting. Nevertheless, in some cases (mainly in the late summer cropping season) wilt may develop and result in yield losses even in methyl bromide-fumigated crops.

Methyl bromide is expected to be banned from use within the next 10 years (in the United States by the year 2001 and in Israel and elsewhere by the year 2010; 23). Accordingly, alternative measures for disease suppression are urgently needed. It is possible that some fungicides may be effective against *M. cannonballus* but, to the best of our knowledge, such efficacy has not yet been identified. In general, control of soilborne pathogens with fungicides is more complicated and less successful than chemical control of foliar diseases (24). The behavior of a fungicide in soil depends on the physical, chemical, and biological properties of both the soil and the fungicide used. Processes such as sorption, degradation, mobility, penetration into the host tissue, and translocation within the plant determine the activity of a compound and its residual life in soil (24). The objective of the present study was to identify fungicides with high efficacy against *M. cannonballus* in culture and to test their activity under field conditions. A brief report with partial results has been published (4).

MATERIALS AND METHODS

Inhibition of *M. cannonballus* in culture. The activity of 29 different fungicides against *M. cannonballus* was determined in vitro (Table 1). Sterile aqueous solutions of the fungicides were mixed with melted yeast extract-glucose agar medium to give a concentration of 10 µg a.i./m. A 12-mm disk of agar with *M. cannonballus* mycelia from a 10-day-old culture was placed in the center of the petri dishes containing the compound-amended agar. The dishes were incubated at 27°C for 5 days, after which the colony diameter was measured in two perpendicular directions and its area calculated. The inhibition of mycelial growth was determined in relation to that of the control (without fungicide) treatment. There were four replicates (petri dishes) for each compound and the test was repeated once. Fungicides that were highly effective in this set of experiments were used in further tests. In the second set of trials, dose-response curves were used to determine the effective dos-

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ages that reduce mycelial growth by 50% (ED₅₀). A series of decreasing concentrations of the fungicides were prepared in water. Yeast extract-glucose agar was amended with the test compounds at concentrations ranging from 10 to 0.01 µg a.i./ml. ED₅₀ was calculated from the regression equation of the log-probit transformed dose-response curves. There were four replicates (petri dishes) for each compound concentration and the test was repeated once.

Management of *M. cannonballus* in field tests. The efficacy of the fungicide fluazinam (Trade name-Frowncide, Ohayo, 50% soluble concentrate, produced by ISK, Japan) in suppression of *M. cannonballus* was determined in three field experiments conducted in the 'Arava region of Israel. The first experiment was conducted in spring 1996 (transplanted in February) and the second and third in late summer (transplanted in the September cropping of 1996 and 1997). These are the two common cropping seasons for melon in the 'Arava. In all experiments, the susceptible melon cultivar Galia was used. In the first experiment, the soil was infested artificially. Plastic cylinders with open ends, 30 cm in diameter and 35 cm long, were filled with field soil and buried flush with the soil surface in beds. The distance between cylinders within rows was 50 cm and intra-row spacing was 160 cm. Drip irrigation tubes were placed on the beds and the beds

were then covered manually with plastic sheets (Polyon, Barqay, Israel). Methyl bromide (Dead Sea Bromine Group, Be'er Sheva', Israel) was applied through the irrigation tubes at a rate of 50 g/m², employing a hot gas method (11). After fumigation, the soil content of the cylinders was infested with *M. cannonballus*. An isolate of *M. cannonballus* obtained from a wilted melon plant was grown for 10 days on potato dextrose agar (PDA) at 27°C. The content of one petri dish culture was blended with 100 ml of sterile water and mixed with 10 liters of the methyl bromide-treated soil taken from the cylinders. In addition, 5 g of a mixture of soil and inoculated oats, prepared according to Mertely et al. (17), was added to the same soil. After infestation, the soil was returned to the cylinders.

Melon plants were transplanted on 13 February 1996, two plants per cylinder. Fluazinam (0.25 g a.i./ml.) was applied to each cylinder as a soil drench in 500 ml of water. The experiment consisted of four treatments: (i) untreated control; (ii) application of fluazinam once, 2 days after planting; (iii) application of fluazinam once, 64 days after planting; and (iv) application of fluazinam twice, 2 and 64 days after planting. Each experimental plot consisted of eight cylinders in one row. There were four replicates and treatments were arranged in a randomized block design. The crop was irrigated, fertilized, and maintained according to the recommendations for melon production in the 'Arava region.

The second and third experiments were conducted in naturally infested soil at the 'En Tamar Experiment Station, located at the northern part of the 'Arava. Melon plants were transplanted on 17 September 1996 and 15 September 1997. Intra-row spacing was 50 cm and beds were 190 cm apart. The second experiment consisted of five treatments: (i) untreated control; (ii) application of fluazinam at a rate of 750 g a.i./ha at 12, 24, 36, and 48 days after planting; (iii) application of fluazinam at a rate of 1,500 g a.i./ha at 12, 24, 36, and 48 days after planting; (iv) application of fluazinam at a rate of 1,500 g a.i./ha starting 7 days after planting and continuing at 7-day intervals for a total of eight applications; and (v) application of fluazinam at a rate of 1,500 g a.i./ha twice, 12 and 48 days after planting. Fluazinam was applied in all treatments as a soil drench in 400 ml of water/plant. Each experimental plot was 5 m long; there were four replicates per treatment, and treatments were arranged in a randomized complete block design.

In the third experiment, plots were either not treated or treated with fluazinam at a rate of 750 g a.i./ha, four times during the season. The fungicide was applied through the drip irrigation system starting 10 days after transplanting and continuing at bi-weekly intervals. Experimental plots were

7 m long and consisted of 14 transplants per plot. There were four replicates per treatment and experiments were arranged in a randomized complete block design.

Wilt was evaluated visually in all experiments. A plant was considered dead when the whole plant exhibited irreversible wilt symptoms. The number of wilted plants and the total number of plants per plot were used to calculate the incidence of wilting (percent). In the first and third experiments, wilt incidence was recorded visually upon fruit ripening. In the second experiment, wilt was evaluated 30, 42, 47, and 57 days after planting. In addition, the presence of *M. cannonballus* in roots of wilted plants from the untreated plots was confirmed. Roots of wilted melon plants were stored in a paper bag in the lab for 3 weeks. The presence of *M. cannonballus* perithecia was visually evaluated.

Mobility of fluazinam in soil. The mobility of fluazinam in soil was evaluated in samples taken from the field and in vitro tests. Soil samples were taken from treatment number iv of the second experiment, 60 days after planting (after seven applications of fluazinam). Soil cores were obtained by inserting a metal tube (4 cm in diameter and 25 cm long) into the moist soil at a distance of 10 cm from a plant and removing it intact with the soil sample. The cores were brought to the laboratory where they were frozen (-18°C) until analysis. The cores were extruded from the metal tubes after minimal defrosting by applying air pressure to the tube. The cores were then sectioned and sub-samples taken for determination of moisture content and fungicide. Soil samples were extracted with water and ethyl acetate in a 1:1 ratio (4 ml each) overnight on a reciprocal shaker. After centrifugation to separate the phases, the organic phase was removed and stored in autosampler vials containing Na₂SO₄ to remove any residual water. The samples were analyzed on a Perkin-Elmer Autosystem gas chromatograph equipped with a nitrogen-phosphorus detector. A standard curve of fluazinam was used for quantitation.

The mobility of fluazinam in soil also was tested in vitro using a thin-layer chromatography (TLC) method (8). Clean glass plates (20 by 20 cm) were coated with sieved (approximately 0.25 mm) 'Arava soil, prepared as a 2:1 soil:water slurry. A TLC applicator was used for the coating procedure, producing a soil layer approximately 0.75 mm thick. Soil TLC plates were air dried and fluazinam solution was applied at marked spots 1.5 cm above the plate base. The final rate of fluazinam at each spot was 0.5, 5, and 50 µg. The plates were placed in a closed glass chamber containing distilled water and with a water-saturated atmosphere. The plate was removed from the chamber and air dried when the water front reached a height of 10 cm. Spores of the fungus *Cladosporium*

Table 1. In vitro growth inhibition of *Monosporascus cannonballus* by 29 fungicides at 10 µg a.i./ml^a

Fungicide tested	Growth inhibition (%)
Benomyl	98.4
Carbendazim	95.7
Carbendazim + diethofencarb	52.1
Dichlofluanid	66.5
Dicloran	40.4
Dimiconazole	77.5
Fenarimol	66.5
Flutriafol	0
Fluazinam	100
Flusufamid	40.4
Hexaconazole	77.5
Imazalil sulfate	66.5
Iprodione	66.5
Kreoxin methyl	100
Maneb	83.2
Mertiram	93.1
Myclobutanil	52.1
Oxycarboxin	40.4
Paclobutrazol	40.4
Polyoxin AL	0
Polyoxin B	66.5
Quinomethionate	83.2
Quintozene	66.5
TCMTB	89.8
Thiophanate methyl	66.5
Tolclofos methyl	83.2
Tolclofos methyl + thiram	0
Triadimenol	0
Uniconazole	0

^a Mycelial growth inhibition was obtained on fungicide-amended medium.

sp., found to be sensitive to fluazinam in preliminary tests, served as a bioassay organism and were sprayed on the soil TLC plates. Spore suspensions were prepared by flooding a *Cladosporium* sp. culture with 10 ml of potato dextrose broth and scraping the medium surface with a glass. The suspension was filtered through eight layers of cheesecloth before use. After spraying the *Cladosporium* sp. spores, the TLC plates were incubated in a moist chamber at 25°C in the dark for 24 h. Inhibition zones of *Cladosporium* sp. growth were used to indicate the location of the fungicide.

Statistical analysis. Data were analyzed statistically using the Minitab software package (Minitab Inc., State College, PA). Results recorded in the field experiments were subjected to analysis of variance (ANOVA) and, when differences were significant at $P \leq 0.05$, Fisher's protected least significant difference (LSD) test was employed. Fungicide dose-response and fluazinam mobility in soil were analyzed by simple regression analysis.

RESULTS

Inhibition of *M. cannonballus* in culture. Of the 29 fungicides tested, fluazinam and kresoxim methyl were the most effective and totally inhibited the growth of *M. cannonballus* in culture at concentrations of 10 µg a.i./ml (Table 1). Results of a second experiment corroborated these results. Since fluazinam also was effective in inhibition of *P. aphanidermatum* in vitro (ED_{50} 0.33 µg a.i./ml), and kresoxim methyl was not (unpublished data), fluazinam was chosen for further tests. *P. apha-*

nidermatum also may be involved in the sudden wilt syndrome, solely or in interaction with *M. cannonballus* (20,21). The ED_{50} of fluazinam for *M. cannonballus* was 0.09 µg a.i./ml.

Management of *M. cannonballus* in field tests. Significant reduction in wilt incidence was achieved in the spring experiment in all plots treated with fluazinam (Fig. 1). One application of the fungicide, either 2 or 64 days after planting, reduced wilt incidence ($P \leq 0.05$) by 59.6 and 46.8%. Application of fluazinam twice (2 and 64 days after planting) resulted in the highest wilt suppression (86.2% wilt reduction). The fungus *M. cannonballus* was isolated from roots of all sampled wilted plants, indicating that *M. cannonballus* was the cause of the sudden wilt syndrome in that trial.

In the second experiment, fluazinam was applied to a late summer cropping of melon transplants grown in a naturally infested field. Although wilt suppression was significant in some of the treatments, its magnitude was less pronounced than in the first experiment. In plots treated four times during the growing season, doubling the rate of fluazinam (from 750 to 1,500 g a.i./ha in each application) did not improve wilt suppression. By the end of the experiment, wilt incidence in the treated plots did not differ significantly from that of the untreated plots (Fig. 2). Increasing the frequency of applications and the resultant total number of applications (from two to four and eight applications during the growing season) improved wilt suppression at the early stages of the epidemic but, by the end of the season, there were no differences in wilt in the different fungicide-treated plots (Fig. 3).

In the third experiment, the efficacy of fluazinam was confirmed. Wilt was observed soon before ripening. In the fluazinam-treated plots, wilt incidence was 11.0%, significantly lower than in the untreated plots, where wilt incidence was 93.7%.

Mobility of fluazinam in soil. The mobility of fluazinam was studied in the laboratory using the soil TLC method and by analyzing soil samples taken from the second fields experiment. Both methods revealed that fluazinam mobility in soil was limited. Inhibition zones of *Cladosporium* sp. (the bioassay organism) on the soil TLC plates were restricted to the area of fungicide application (Fig. 4). In soil sampled from the field, exceedingly high concentrations of fluazinam were found to a depth of 4.5 cm, after which the concentration of fluazinam diminished rapidly and only 5 mg/kg soil was recovered at a depth of 25 cm (Fig. 5).

DISCUSSION

The widespread and destructive nature of sudden wilt of melons caused by *M. cannonballus* constitutes a serious threat to melon cultivation in the major production areas of the world. Currently, the only means to control the disease in Israel is application of methyl bromide as a pre-plant soil treatment (26). The use of methyl bromide will be banned worldwide within the next 5 to 10 years (23). Thus, the development of alternative control measures is of great importance to the melon industry. Application of fungicides to the soil is a possible solution, but application prior to planting (as soil disinfestation) is more common and successful than application of fungicides during the growing season (24).

Fig. 1. Effect of the fungicide fluazinam on sudden wilt of melons caused by *Monosporascus cannonballus*. The experiment was conducted on artificially inoculated plants in the spring. Fluazinam (0.25 g a.i./two plants) was applied as a soil drench in 500 ml of water at 2, 64, or 2 and 64 days after transplanting. Bars followed with common letter are not significantly different ($P = 0.05$) as determined by Fisher's protected least significant difference test.

Fig. 2. Effect of the rate of the fungicide fluazinam on sudden wilt of melons caused by *Monosporascus cannonballus*. The experiment was conducted in the late summer season of 1996 under natural epidemics. The fungicide was applied as a soil drench (400 ml/plant), at two rates, 750 and 1,500 g a.i./ha, at 12, 24, 36, and 48 days after planting. Vertical bars represent the least significant difference (at $P \leq 0.05$) as determined by Fisher's protected least significant difference test.

Fig. 3. Effects of the number of fluazinam applications on *Monosporascus cannonballus*. The experiment was conducted in the late summer season of 1996 under natural epidemics. The fungicide was applied as soil drench (400 ml/plant), at a rate of 1,500 g a.i./ha at 12, 24, 36, and 48 days after planting. Vertical bars represent the least significant difference (at $P < 0.05$) as determined by Fisher's protected least significant difference test.

Fig. 4. Soil thin layer chromatography (TLC) plates used for determining the mobility of the fungicide fluazinam (trade name "Frownicide") in soil. Fluazinam was applied at three rates (0.5, 5, and 50 µg) to soil taken from the 'Arava region in southern Israel. TLC plates were sprayed with spores of a *Cladosporium* sp., which was used as a fungal bioassay for detecting the presence of the fungicide.

Fungicide application to existing crops is practiced mainly for management of seedling diseases such as *Pythium* and *Rhizoctonia* damping off. In these pathosystems, host-pathogen interaction occurs during a short period of time and in a relatively limited soil volume. Chemical control of pathogens that attack the deep root system or older plants, such as *Fusarium*, *Verticillium*, and *Monosporascus* spp., is less common, due to the complexity of the interaction. To date, practical experience for this type of pathosystem is lacking (5).

Fluazinam and kreoxim methyl both completely inhibited vegetative growth of *M. cannonballus* in culture. The in vitro tests involved inhibition of fungal mycelia. Fluazinam was chosen for further testing under field conditions because it was also effective against *P. aphanidermatum*. In the field, *M. cannonballus* ascospores are the primary source of infection, not mycelia. Ascospores germinate only in the rhizosphere of roots of susceptible melon cultivars in response to exudates secreted by the root (25); therefore, it was not possible to test the activity of the various fungicides in vitro on ascospores.

Fluazinam efficacy in suppression of sudden wilt was confirmed in the field, but results varied. In the first and third experiments, control at fruit ripening was approximately 87%; whereas, in the second experiment, it was only 32%. Variation in control may result from differences in inoculum load in soil, growing conditions, and temperature regimes that prevail during the season. Analysis of experiments conducted in the 'Arava region revealed that wilt incidence is enhanced at high temperatures, especially upon ripening, where the crop may suffer from temporal water stresses (3,20). Similarly, Kim et al. (10) reported that, in Arizona, infections developed earlier and wilt intensity was more severe in the late summer cropping (planted in July) than in spring cropping (planted in January to February). They also indicated that severe epidemics were associated with soil temperatures greater than 25°C (10).

Fluazinam efficacy also was demonstrated in a commercial field (28). The fungicide was applied via the drip irrigation system to part of a field (0.3 ha in size) located in 'En Tamar in the northern 'Arava region. Applications were made four times (18, 33, 47, and 61 days after planting) at a rate of 750 g a.i./ha. Although the entire field was fumigated prior to planting with methyl bromide, 94% of the plants had collapsed by the end of the season in the part that was not treated with fluazinam. In the part of the field that was treated with fluazinam, wilt incidence was 4%. As indicated above, methyl bromide does not always provide adequate disease suppression in commercial situations.

Soil TLC and field soil analysis revealed that fluazinam mobility in soil is limited

Fig. 5. Fluazinam distribution in field soil profile. Samples were taken from late-summer experiments. Fluazinam was applied as drench seven times, at a rate of 1,500 g a.i./ha.

(Figs. 4 and 5). When applied in water, most of the fungicide is absorbed in the soil surface layer, resulting in a zone of high rate of the compound, and decreasing concentrations with depth and distance from the application site. Nevertheless, rates measured even at a depth of 25 cm were sufficient to control *M. cannonballus* (Fig. 5). It may be possible that applications that result in a more even distribution of the fungicide in soil would further improve control efficacy.

The present study shows, for the first time, that sudden wilt caused by *M. cannonballus* may be managed by post-planting applications of a fungicide. However, control efficacy was inadequate in some cases and varied among experiments; therefore, more research is needed before fluazinam can be used commercially for disease suppression. Nevertheless, even when the rate and application method of fluazinam are optimized, it should not be used as a sole measure for disease suppression. Instead, it should be incorporated as a component in an integrated management approach. Such an approach also may involve the use of resistant or tolerant melon cultivars, transplanting of grafted melon plants, and reduction of initial inoculum load in the soil.

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