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Systemic fungitoxic action of benomyl against Fusarium oxysporum f. sp. melonis in vivo

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Abstract

It is demonstrated that benomyl, applied as a soil drench, is highly efficient in reducing the quantity of mycelium in muskmelon plants infected by *Fusarium oxysporum* f. sp. *melonis*. When treated before symptoms appear, plants are generally cured. The blending technique used for assessing fungal spread in the xylem vessels is particularly convenient for studying the action of a systemic fungicide in a plant with vascular wilt.

Introduction

Benomyl, methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate, is a new systemic fungicide, both preventive and curative. Its efficiency against a great number of fungi when applied to the aerial parts of plants has been demonstrated in both greenhouse and field experiments (Delp and Klopping, 1968; Bourdin et al., 1969; Engelhard, 1969; Evans et al., 1969; McIntosh, 1969; Waffelaert, 1969). Its systemic fungicidal effect, when applied by seed soaking or soil drenching, has been reported in the control of powdery mildew (Hammett, 1968; Schroeder and Provvidenti, 1968; Gilpatrick, 1969; Cole et al., 1970; Johnston, 1970), *Ustilago* spp. (Halisky et al., 1968; Hardison, 1968; Metcalfe and Brown, 1969; Tyler, 1969) and various root rot pathogens (Al-Beldawi and Pinckard, 1968; Lafon and Bugaret, 1969; Papavizas et al., 1970; Saenger, 1970).

When applied to the soil, benomyl also prevents *Verticillium* wilt of cotton (Erwin et al., 1968) and tomato (Ebben and Last, 1969) and *Fusarium* wilt of tomato (Biehn and Dimond, 1969) after inoculation either of stems or roots with spores. On naturally infested soil Wensley (1969) observed a reduction of *Fusarium* wilt of muskmelon after seed treatment.

In these reports on vascular wilts the effect of benomyl has been estimated on the basis of the reduction or prevention of disease symptoms on the plant. Another important factor in testing a systemic fungicide is its action on the parasite in vivo.

This paper reports the action of benomyl on the quantitative development of the parasite in stems of muskmelon plants (*Cucumis melo* L.) inoculated with *Fusarium oxysporum* Schlecht. f. sp. *melonis* Snyd. & Hans.

Material and methods

Muskmelon plants (Variety 'Noir des Carmes') were grown in 450 ml pots, on partially sterilized compost, in an air-conditioned greenhouse at 29° C during the day and 24° C during the night.

At the stage of two fully developed leaves the plants were inoculated by injecting into the soil 20 ml of a spore suspension (15×10^6 conidia/ml) of F. *oxysporum* f. sp. *melonis*. Benomyl was applied by drenching, per pot and per application, 150 mg of the commercial wettable powder Benlate (50 % a.i.).

The treatments examined included: one application 3 days before inoculation (A); one application 3, 7 or 11 days after inoculation on plants which did not yet show any symptoms (B, C, D); one application 11 days after inoculation on plants already showing first symptoms (E); untreated inoculated control (F).

Ten days after inoculation the first symptoms began to appear in some of the untreated plants. For this reason we treated and analysed separately on the eleventh day plants without any symptoms (D) and plants showing the first signs of yellowing on leaves (E). Treatments A, B, F each comprised 50 plants and C, D and E 40 plants.

The quantity of mycelium in the host stems was estimated by a blending technique (Stover and Waite, 1953) 7, 11, 13 and 29 days after inoculation. Each determination was made using a sample comprising 10 stems. The stems were surface sterilized for 2 min. in a 1,5 % sodium hypochlorite solution, washed, placed in a blender with sterile water (10 to 20 g fresh weight in 100 ml water) and the homogenate plated after serial dilutions.

Results and discussion

The quantities of living mycelium of F. *oxysporum* f. sp. *melonis* in the stem tissues after the various treatments are reported in Table 1. These results were confirmed by two similar experiments.

One application of benomyl before inoculation or three days after inoculation restricted stem colonization to a very low level and the mycelium was often even not detectable.

Applications seven and eleven days after inoculation to plants without symptoms rapidly reduced the quantity of living mycelium within the plants, demonstrating the strong curative effect of benomyl. However, 3 of the 20 plants showing no symptoms and treated eleven days after inoculation, were dead on the twenty-ninth day, 6 had shown slight wilting immediately after the application of benomyl but had rapidly recovered, while the remainder never exhibited any symptoms of the disease.

Of the 20 plants already showing symptoms on the day of the treatment, 12 died and 8 were cured.

The time when symptoms appear thus seems to be critical in determining the effect of the treatment, at least in our experiments.

Horst and Hoitink (1968) were able to control infection of *Cylindrocladium* sp. in *Azalea* sp. by drenching the soil with benomyl 5 days after inoculation. Erwin (1969), on the other hand, failed to control the onset of cotton wilt by applying the substance more than 3 to 5 days after inoculation with a virulent isolate of *Verticillium albo-atrum*.

Table 1. Numbers of colonies of *F. oxysporum* f. sp. *melonis* per g fresh weight of blended stems, at different days after inoculation and after various treatments with benomyl.

Treatment	Number of days between inoculation and treatment	Observed numbers of colonies/g fresh weight, at various days after inoculation			
		7	11	13	29
A	–3 (1)	28	nd (2)	4	nd
B	3	37	6	4	16
C	7	28,200	1,120	139	20
D	11	28,200	124,000	219 (3)	26 (3)
E	11 symptoms (4)	28,200	338,000	539,000	465 (5)
F	untreated control	28,200	1,930,000	6,450,000	—

(1) Treated three days before inoculation.
(2) nd: not detectable in 5 ml of the homogenate.
(3) Determined only on plants without symptoms.
(4) Treatment on plants showing the first symptoms.
(5) Determined in the stems of the surviving plants.

Tabel 1. Aantal kolonies van F. oxysporum f. sp. melonis per g vers gewicht van gemalen meloenstengels, op verschillende dagen na de inoculatie en voor de diverse behandelingen met benomyl.

The high fungitoxic effect of benomyl on F. *oxysporum* in the plant differs from its fungistatic activity observed in vitro with shaken cultures (Maraite and Decallonne, 1970).

All the treated plants showed, even 8 weeks after treatment, some stunting and slight toxicity symptoms, characterized by a marginal chlorosis on young leaves as well as on older ones.

After harvesting the first planting, a second planting and inoculation were done in the same benomyl treated soil.

The young muskmelons showed some symptoms of phytotoxicity 14 days after replanting but were still protected against *Fusarium*. These observations confirm the persistence of benomyl in the soil (Hine et al., 1969; Jacobsen and Williams, 1969).

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Samenvatting

Systemische fungicide werking van benomyl op Fusarium oxysporum f. sp. melonis in vivo

Met behulp van een mixertechniek wordt aangetoond, dat na een begieting van de wortels van jonge meloenen met benomyl (75 mg actieve stof/pot van 450 ml) de infektie door *Fusarium oxysporum* f. sp. *melonis* in kunstmatig besmette aarde heel laag blijft. In geïnfekteerde planten veroorzaakt deze behandeling een sterke vermindering van de hoeveelheid *Fusarium* in de stengel. Wanneer de planten behandeld worden

juist voor zij de eerste symptomen aantonen, wordt gewoonlijk de ontwikkeling van de parasiet gestopt zodat de planten kunnen genezen. Het produkt blijft tenminste 10 weken actief in de grond.

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