Suppression of Fusarium Wilt of Carnation in a Composted Pine Bark and a Composted Olive Pumice

J. PERA and C. CALVET, Departament de Patologia Vegetal, Institut de Recerca i Tecnologia Agroalimentàries, Centre d'Investigació Agrària de Cabrils, 08348 Cabrils, Spain

ABSTRACT

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The use of a composted olive pumice or a commercial composted pine bark instead of sphagnum peat in container media delayed the appearance of wilt caused by *Fusarium oxysporum* f. sp. *dianthi* in cultivar Lena carnations. After 5 mo, wilt incidence in media with composted olive pumice and composted pine bark with an initial inoculum density of 10^4 cfu/cm^3 was 20 and 40%, respectively, whereas in a similarly infested medium with sphagnum peat as the organic component, wilt incidence was 99%.

Vascular wilt incited by *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. & Del.) Snyd. & Hans. causes great damage in all areas of Spain where carnations are grown. Integration of proper cultural and control practices, such as soil disinfestation and the use of partially resistant cultivars, may reduce the incidence of vascular wilt (3), but these practices do not provide sufficient control. Difficulties encountered in control of Fusarium wilt have stimulated the search for biocontrol systems. Suppressiveness of several soils and substrates has been investigated (1,15,19,20).

Soilless substrates for propagation of carnation stem cuttings and for production of plants on raised benches have traditionally been prepared with peat and inorganic materials such as sand or expanded clays. Despite some exceptions (18), these substrates generally are conducive to diseases caused by soilborne pathogens (2,19), including *F. oxysporum* (5). The use of organic substrates prepared with composted residues is becoming a general practice for the production of ornamentals in containers. Some of these composts are suppressive to plant pathogens (2,9), and their use has reduced or eliminated fungicide or sterilization treatments (8).

The aim of this work was to compare the development of Fusarium wilt of carnation in soilless media prepared with sphagnum peat, composted pine bark, or composted olive pumice.

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MATERIALS AND METHODS

Container media. Organic substrates used in the preparation of container media were sphagnum peat (Floratorf-500, Floratorf, Torfstrenverband GmbH, D-2900 Oldenburg, Germany), pH 3.8; composted pine bark (IMC-2, Bark Products Ltd., Via Augusta 13-15, Barcelona, Spain), pH 7.1; and a composted olive pumice, pH 7.4 (4,13,14). They were mixed separately (1:1, v/v) with steamed sand (80 C, 60 min). The pH of the peat-sand mixture was adjusted to 6.8 with $CaCO_3$.

Inoculum. The strain of *F. o.* f. sp. *dianthi* race 2 (7) used throughout this work was isolated from wilted carnations from a commercial greenhouse in Cabrils, Catalonia (northeastern Spain). Inoculum was produced on a medium consisting of chopped carnation leaves mixed with perlite (Europerl, Dicalite española s.a., Beethoven 1-31, Can Jardi, Rubi, Barcelona, Spain) (1:1, v/v). This mixture was distributed as 750-ml volumes into 1-L flasks. The moisture level was adjusted to 50% (w/w) with distilled water. Flasks were autoclaved (120 C, 60 min) twice, with a 24-hr interval. Five 5-mm-diameter agar disks recovered from fresh fungal plate cultures on Difco potato-dextrose agar (PDA) were added to each flask. Flasks were then incubated at 25 C and shaken every 3 or 4 days to distribute fungal growth uniformly throughout the medium. After 28 days, the cultures were spread as thin layers on trays and left to dry at 25 C for 5 days. Dried cultures were mixed with sterile perlite (1:10, v/v), ground, passed through a 1-mm screen, and stored in a sterile container at 5 C until needed for inoculum. The inoculum density was determined by colony counts of serial dilutions on a selective medium (12). Inoculum (1.8×10^7 cfu/g dry wt) was added to container media, and the final density of the pathogen was adjusted to give 10^3, 5×10^3, and 10^4 cfu/cm^3 of container medium. Uninfested media were used as controls.

Experimental design. Infested and uninfested container media were distributed into rectangular containers (25 × 90 × 20 cm deep) with four replications per container medium and inoculum density. Containers were placed randomly on a greenhouse bench. Ten culture-indexed carnation cuttings (cultivar Lena) were planted in each container during the first week of July. Plants were watered as necessary and were fertilized every second week with 2 L of nutrient solution per container (1.2 g NH_4NO_3, 0.2 g $[NH_4]_2HPO_4$, 0.9 g KNO_3 per 1 L of distilled water). Plants were maintained for 5 mo (July–November) at 24 ± 3 C without supplemental light. The percentage of plants showing symptoms of Fusarium wilt was monitored throughout the 5-mo period, and the mean percentage of wilted plants was calculated after intervals of 2, 3.5, and 5 mo. At harvest time, tissue samples from plants with vascular necrosis were surface-sterilized and plated on PDA to ensure a correct diagnosis. Analysis of variance and multiple range comparisons were employed for processing angular-transformed data.

Effect of sterile water extracts from the substrates on mycelial growth of *F. o.* f. sp. *dianthi*. Before plant growth, 100 ml of each organic substrate used in the container media were shaken vigorously for 30 min in 500 ml of sterile distilled water at 25 C. Suspensions were filter-sterilized (0.15 μm) thereafter. The pH of sphagnum peat had been adjusted previously with $CaCO_3$ to reach a value similar to that of the other media. Therefore, the pH of all extracts was in the range 7.0–7.2. Extracts were mixed with water agar (Difco) at two proportions to prepare culture media: 15 ml of extract + 5 ml of 2% water agar and 10 ml of extract + 10 ml of 1% water agar per petri dish. The final pH of the extracts in water agar was 6.8 ± 0.1. In control treatments, the extract was replaced with the same volume of sterile distilled water. A 5-mm-diameter disk from a fresh culture of *F. o.* f. sp. *dianthi* race 2 on water agar was placed on the center of each plate (three plates per treatment). Fungal growth, expressed as colony diameter, was measured after 5 days of incubation at 25 C.

RESULTS AND DISCUSSION

Suppressiveness of container media. Disease progress curves (Fig. 1) reveal that the incubation period was influenced by the inoculum density as well as the container medium. Symptoms of Fusarium wilt were first observed after 2 mo on plants grown in the peat medium infested with 10^3 cfu/cm^3. At that time, 20 and 30% of plants in media with 5×10^3 and 10^4 cfu/cm^3, respectively, showed wilt. The appearance of symptoms in plants subjected to 10^3 cfu/cm^3 was delayed successively longer in the composted pine bark and composted olive pumice media. The first symptoms were not observed until after 3.5 mo of growth in the composted olive pumice medium, even at the highest inoculum density, whereas the percentages of plants with wilt were 22.5 and 60%, respectively, in the composted pine bark and peat media.

The percentage of plants wilted after 5 mo of growth differed significantly ($P = 0.05$) among container media ($F = 41.39$) and among the inoculum density treatments ($F = 14.57$). There were no significant interactions between these two variables. Diseased plants were not observed in any of the uninfested controls. Incidence of wilt increased with increasing inoculum density in all the container media. The percentages of plants wilted in the composted olive pumice and composted pine bark media were significantly ($P = 0.05$) lower than in the sphagnum peat medium at 5×10^3 and 10^4 cfu/cm^3 (Fig. 1). After 5 mo of growth, the percentages of plants with wilt symptoms in both compost-amended media infested with 10^4 cfu/cm^3 were similar to the percentages registered for the peat medium infested with one-tenth as much inoculum. No wilted plants were detected in composted olive pumice at the lowest inoculum density. This density was similar to the concentration of propagules of *F. o.* f. sp. *dianthi* found in naturally infested sandy soil in Catalonia (J. Pera, *unpublished*).

Effect of sterile extracts on mycelial growth of *F. o.* f. sp. *dianthi*. Fungal growth on solid media supplemented with any of the substrate extracts was not significantly different ($P = 0.01$) from that on water agar. Apparently, composted olive pumice and composted pine bark did not contain water-soluble substances in a concentration high enough to affect development of the pathogen under the experimental conditions. The most toxic inhibitory compounds purified from bark composts are ethyl esters of C_{18} hydroxylated organic acids (10). Although raw olive pumice contains a considerable proportion of phenolic compounds and organic acids, predominantly oleic and linoleic acids, they are all undetectable after 4 mo of composting (6). Therefore, the reduced incidence of Fusarium wilt in this substrate possibly may not have been due to the presence of chemical compounds with a fungicidal activity, such as reported for inhibition of *Phytophthora* spp. in composted bark or sawdust (11,16,17).

Composted olive pumice is a new organic compost showing some suppressiveness to Fusarium wilt of carnation because it delayed the appearance of symptoms. The use of composted olive pumice and composted pine bark media for short-term cropping practices, such as for rooting of stem cuttings and for outplanting of plantlets produced by vegetative propagation in vitro, may reduce losses. Before these media may be used for wilt suppression in carnations grown through the conventional 2-yr production cycle, however, additional research is needed on the incidence of latent infection in nonwilted plants and on the nature of the suppressive effect.

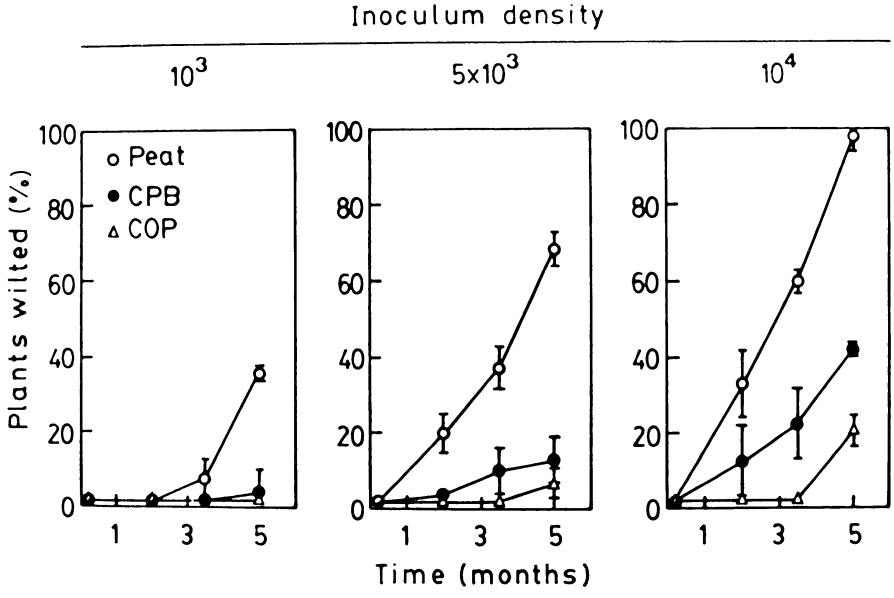

Fig. 1. Disease progress, expressed as mean percentage of plants wilted, in sphagnum peat-sand (peat), composted pine bark-sand (CPB), and composted olive pumice-sand (COP) container media at three inoculum densities (cfu/cm^3 of container medium) of *Fusarium oxysporum* f. sp. *dianthi* race 2. Means of four replicates of 10 plants each. Bars indicate standard deviation.

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LITERATURE CITED

1. Alabouvette, C. 1986. Fusarium wilt-suppressive soils from the Châteaurenard region: A review of a 10-year study. Agronomie 6:273-284.
2. Alabouvette, C., Couteaudier, Y., and Louvet, J. 1982. Comparaison de la réceptivité de différent sols et substrats de culture aux fusarioses vasculaires. Agronomie 2:1-6.
3. Baker, R. 1980. Measures to control Fusarium and Phialophora wilt pathogens of carnations. Plant Dis. 64:743-749.
4. Calvet, C., Pagés, M., and Estaún, V. 1985. Composting of olive marc. Acta Hortic. 172:255-262.
5. Chef, D. G., Hoitink, H. A. J., and Madden, L. V. 1983. Effects of organic components in container media on suppression of Fusarium wilt of chrysanthemum and flax. Phytopathology 73:279-281.
6. Estaún, V., Calvet, C., Grases, J. M., and Pagés, M. 1985. Chemical determination of fatty acids, organic acids, and phenols, during olive marc composting process. Acta Hortic. 172:263-270.
7. Garibaldi, A. 1977. Race differentiation in *Fusarium oxysporum* f. sp. *dianthi* and varietal susceptibility. Acta Hortic. 71:97-101.
8. Hoitink, H. A. J. 1980. Composted bark, a lightweight growth medium with fungicidal properties. Plant Dis. 64:142-147.
9. Hoitink, H. A. J., and Fahy, P. C. 1986. Basis for the control of soilborne plant pathogens with composts. Annu. Rev. Phytopathol. 24:93-114.
10. Hoitink, H. A. J., and Kuter, G. A. 1985. Effect of compost in container media on diseases caused by soilborne plant pathogens. Acta Hortic. 172:191-198.
11. Hoitink, H. A. J., Van Doren, D. M., Jr., and Schmitthenner, A. F. 1977. Suppression of *Phytophthora cinnamomi* in a composted hardwood bark potting medium. Phytopathology 67:561-565.
12. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
13. Pagés, M., Estaún, V., and Calvet, C. 1985. Physical and chemical properties of olive marc compost. Acta Hortic. 172:271-276.
14. Pera, J., Calvet, C., and Camprubi, A. 1986. Succession of microbial populations during olive marc composting. Acta Hortic. 178:271-277.
15. Scher, F. M., and Baker, R. 1980. Mechanism of biological control in a Fusarium-suppressive soil. Phytopathology 70:412-417.
16. Sivasithamparam, K. 1981. Some effects of extracts from tree barks and sawdust on *Phytophthora cinnamomi* Rands. Aust. Plant Pathol. 10:18-20.
17. Spencer, S., and Benson, D. M. 1982. Pine bark, hardwood bark compost, and peat amendment effects on development of *Phytophthora* spp. and lupine root rot. Phytopathology 72:346-351.
18. Tahvonen, R. 1982. The suppressiveness of Finish light coloured sphagnum peat. J. Sci. Agric. Soc. Finl. 54:345-356.
19. Tramier, R., Pionnat, J. C., and Antonini, C. 1983. Alteration on incidence of Fusarium wilt of carnation by suppressive soils. Acta Hortic. 141:89-94.
20. Yuen, G. Y., McCain, A. H., and Schroth, M. N. 1983. The relation of soil type to suppression of Fusarium wilt of carnation. Acta Hortic. 141:95-102.