Verticillium nigrescens from Peppermint

H. A. Melouk and C. E. Horner

Research Associate, Department of Botany and Plant Pathology, Oregon State University, and Plant Pathologist, ARS, USDA, Corvallis 97331, respectively.

Cooperative investigations of ARS, USDA, and the Department of Botany and Plant Pathology, and Technical Paper No. 3397, Oregon Agricultural Experiment Station, Corvallis 97331.

Financial assistance from the Mint Industry Research Council is gratefully acknowledged.

ABSTRACT

Verticillium nigrescens was isolated from peppermint plants showing mild wilt symptoms. Average size of *V. nigrescens* chlamydospore produced on potato-dextrose agar was 5.14 × 6.40 μm. The fungus grew well at 20 to 25 C on Czapek Dox agar and a mint rhizome medium. Inoculation of eight plant species revealed that *V. nigrescens* colonized the roots and was weakly pathogenic to peppermint, spearmint, tomato, eggplant, and pepper.

Phytopathology 64:1267-1268

Additional key words: Mentha, Capsicum, Lycopersicon, Solanum.

Pethybridge (6) in 1919 isolated two chlamydospore-producing *Verticillium* species from potato plants and tubers in England. He named them *V. nigrescens* Pethybr. and *V. nubilum* Pethybr. Devaux and Sackston (1), and more recently Hoes (2), have studied the taxonomy and morphology of *V. nigrescens* in relation to other species of *Verticillium*. *V. nigrescens* and *V. nubilum* can be distinguished on the basis of their growth response to temp and chlamydospore morphology (2).

We isolated a chlamydospore-producing species of *Verticillium* in 1972 from peppermint (*Mentha piperita* 'Mitcham') showing mild symptoms of wilt. The wilted plants were growing in a field of peppermint that was being certified as free from Verticillium wilt. Thus it became important to identify the fungus accurately and to determine its pathogenic potential to commercially grown mint varieties. In this paper we report on: (i) growth response of our isolate of *V. nigrescens* to temp, (ii) chlamydospore production and measurements, and (iii) its pathogenicity to peppermint, spearmint (*Mentha cardiaca* Baker 'Scotch'), and other selected plant species.

Linear growth rate in the dark was determined on Czapek Dox agar (pH 7.2) and a mint rhizome medium (pH 7.2), at temp ranging from 5 to 30 C. Twenty ml of medium were placed in each flat-bottom petri dish and inoculated in the center with 7-mm disks from potato-dextrose agar (PDA), on which the organism had been growing for 3 wk. Radial growth measurements were made 7, 10, and 14 days after inoculation. Mint rhizome medium was prepared by placing 40 g of chopped peppermint rhizomes in a flask with 1.0 liter of distilled water, autoclaving the mixture for 10 min, then straining it through cheesecloth. To 1.0 liter of this infusion, $NaNO_3$, 1.5 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; and agar, 15 g were added, and the mixture was autoclaved for 15 min. Maximum growth on Czapek Dox agar was at 25 C, with satisfactory growth from 20 to 30 C (Fig. 1). On mint rhizome medium, maximum growth was at 20 C, with satisfactory growth from 15 to 25 C (Fig. 1). On both media visible growth

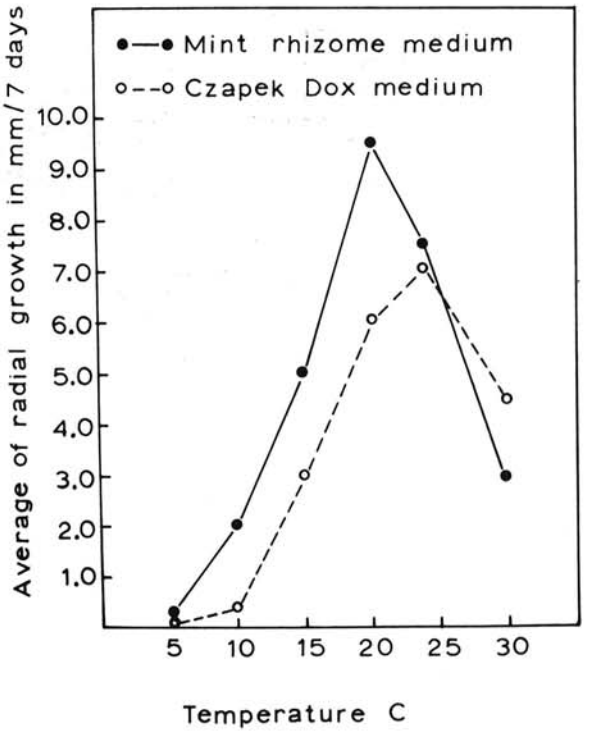

Fig. 1. Effect of temperature on radial growth of *Verticillium nigrescens* on Czapek Dox agar and mint rhizome medium.

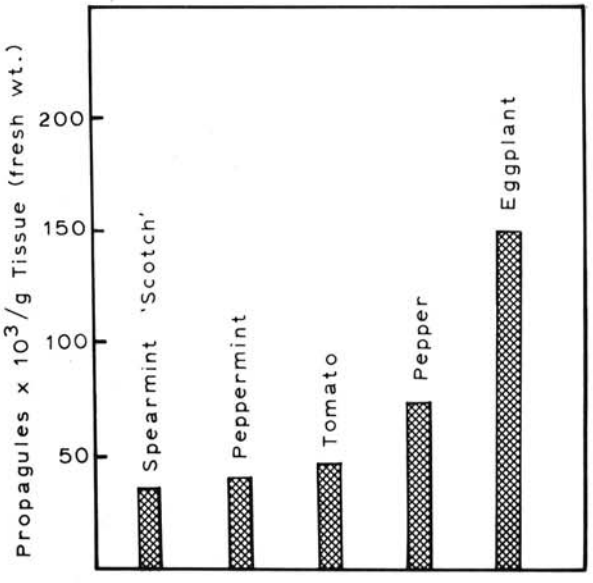

Fig. 2. Propagules of *Verticillium nigrescens* from plant roots 10 wk after inoculation.

was detected at all temp within 7 days after inoculation, except at 5 C, where no growth was found.

V. nigrescens produced chlamydospores after 2 wk of growth in the dark at 20 C on both Czapek Dox agar and mint rhizome media. The majority of the chlamydospores were oval, but a few were almost round. They were borne terminally or intercalarily, singly or in chains. Average size of 100 chlamydospores produced on PDA was 5.14 × 6.40 μm, with a range of 4-6 × 4-8 μm. These data on chlamydospore size and growth response to temp, in comparison with data of others (2, 3), clearly indicate that our isolate is *V. nigrescens.* We believe this to be the first report that *V. nigrescens* has been recovered from diseased plants in Oregon.

For pathogenicity tests, seedlings (5 wk old) of tomato (*Lycopersicon esculentum* Mill. 'Bonny Best'), pepper (*Capsicum frutescens* L. 'California Wonder'), potato (*Solanum tuberosum* L. 'Russet Burbank'), eggplant (*Solanum melongena* L. 'Black Beauty'), alfalfa (*Medicago sativa* L. 'Vernal'), beans (*Phaseolus vulgaris* L. 'Tenderette'), and rooted shoot-tip cuttings of Mitcham peppermint and Scotch spearmint were inoculated by dipping the roots for 60 min in a conidiospore suspension (10^6 spores/ml). Spores were obtained from cultures grown on PDA for 3 wk. Three replications of six plants each for each species were used. After inoculation, test plants were transplanted to pots containing a mixture of sandy loam soil and sand (2:1, v/v). The plants were kept on a greenhouse bench at 21-23 C. Daylight was supplemented by fluorescent lights to provide a 15-h daylength.

Four to five wk after inoculation, 75% of the inoculated Mitcham peppermint and Scotch spearmint plants showed wilt symptoms, consisting of twisting of the top leaves, epinasty, and general stunting, similar to those observed in the field. At 6-8 wk after inoculation, these symptoms started to disappear, and the plants resumed normal growth. Pepper, eggplant, and tomato showed general stunting and chlorosis 4-6 wk after inoculation, but later the plants recovered. There were no symptoms on inoculated potato, bean, or alfalfa. Attempts to recover the fungus from stems of inoculated plants 4 and 6 wk after inoculation failed. The degree of root colonization was quantitatively assessed for each test species 10 wk after inoculation. Root samples were washed for 16 h in running cold tap water, finely fragmented, diluted, and plated on a selective medium (4). Colonies were counted after 2 wk incubation in darkness at 20 C. Each colony of *V. nigrescens* was considered to have grown from a single propagule. The large numbers of propagules detected from the roots of peppermint, spearmint, eggplant, tomato, and pepper (Fig. 2) indicated that roots of these species were colonized by the fungus. Root colonization was not detected on potato, bean, and alfalfa. Skotland (7) isolated *V. nigrescens* from sweet cherry and spearmint in Washington State and found it to be nonpathogenic to peppermint, eggplant, cantaloupe, and tomato. However, Isaac (3) reported that two isolates of *V. nigrescens* from soil were pathogenic to tomato, potato, eggplant, and hops. While a strains of *V. dahliae* Kleb. [= *V. albo-atrum* Reinke & Berth. var. *menthae* Nelson (5)] causes a very serious disease of Mitcham peppermint and Scotch spearmint, our results suggest that *V. nigrescens* is not a serious pathogen of those plants.

LITERATURE CITED

1. DEVAUX, A. L., and W. E. SACKSTON. 1966. Taxonomy of Verticillium species causing wilt of horticultural crops in Quebec. Can. J. Bot. 44:803-811.
2. HOES, J. A. 1971. Development of chlamydospores in Verticillium nigrescens and V. nubilum. Can. J. Bot. 49:1863-1866.
3. ISAAC, I. 1949. A comparative study of pathogenic isolates of Verticillium. Trans. Br. Mycol. Soc. 32:137-156.
4. NADAKAVUKAREN, M. J., and C. E. HORNER. 1959. An alcohol agar medium selective for determining Verticillium microsclerotia in soil. Phytopathology 49:527-528.
5. NELSON, R. 1950. Verticillium wilt of peppermint. Mich. Agric. Exp. Stn. Tech. Bull. 221. 259 p.
6. PETHYBRIDGE, C. H. 1919. Notes on some saprophytic species of fungi associated with diseased potato plants and tubers. Trans. Br. Mycol. Soc. 6:104-120.
7. SKOTLAND, C. B. 1971. Pathogenic and nonpathogenic Verticillium species from south central Washington. Phytopathology 61:435-436.