Growth in Culture and Pathogenicity of Phoma strasseri to Peppermint

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ABSTRACT

Phoma strasseri, the causal agent of rhizome and stem rot of peppermint, grew best in culture at 20 to 25 C. Starch was the best carbon source for growth of *P. strasseri,* but good growth was also obtained with fructose. Disease development was more rapid in wounded than in nonwounded inoculated peppermint stems. As stems matured, they became more resistant to disease development. In young rhizomes, disease developed over a broad range of temperatures, but was most rapid at 20 to 25 C. Root inoculation resulted in extensive infection followed by stunting and reddening of the plants.

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A disease of peppermint (*Mentha piperita* L. 'Mitcham') caused by *Phoma strasseri* Moesz occurs in Western Oregon and Washington. We formerly called the causal agent *P. menthae* Strasser (5, 6), but recent information indicates that the correct name is *P. strasseri* (3). Apparently a similar disease of mints occurs in Japan (4). The disease is characterized by black lesions and cankers on stems and rhizomes. On young shoots, infection may progress rapidly and cause a condition similar to postemergence "damping off" disease of other plants. This paper reports studies on the growth of *P. strasseri* in culture, effect of temperature on disease development, and other basic information relating to pathogenicity.

MATERIALS AND METHODS, RESULTS.—*Temperature and linear growth in culture.*—Effects of temperatures ranging from 5 to 35.5 C on *P. strasseri* growing in the dark on Czapek-Dox agar (pH 7.3) were determined in flat-bottom petri plates containing 20 ml of medium. The medium in petri plates was inoculated in the center with discs of Czapek-Dox agar medium 4 mm in diam on which the organism had been growing for 2 weeks. Radial growth in mm was recorded daily for 7 days. Optimum temperature for growth was 25 C, with good growth from 15 to 25 C (Fig. 1). Little growth occurred at 5 and 30 C, and no growth was observed at 35.5 C. At all temperatures tested, visible growth occurred within 3 days after inoculation, except at 5 and 35.5 C where no growth was detected.

Growth of P. strasseri on various carbon sources.—The following carbon sources were tested: glucose, fructose, galactose, maltose, lactose, sucrose, and starch. Twenty g of the carbon source were added to each 1 liter basal medium, which consisted of $NaNO_3$, 3 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeCl_2 \cdot 4H_2O$, 0.01 g; and biotin, 5 mg. Media were sterilized by filtration, except for starch, which was autoclaved and added separately as powder.

Phoma strasseri was grown on the various carbon sources in 250-ml Erlenmeyer flasks, each containing 50 ml of medium. Flasks were inoculated with 1 ml of a spore suspension (200,000 spores/ml) obtained by scraping spores from a culture grown on Czapek-Dox agar. Spores were washed twice with sterile distilled water, then resuspended in sterile distilled water. Flasks were incubated at 25 C under continuous light (600 ft-c) provided by fluorescent tubes. Noninoculated control flasks were included.

Harvests were made of four replicate flasks each after 8, 14, and 18 days of incubation. Dry weight of mycelia and spores was the criterion of growth. Spores and mycelia were removed from the media by filtration on fiberglass pads, then dried in an oven for 2 hr at 110 C. Starch was the best carbon source for growth of *P. strasseri* (Fig. 2). However, the fungus grew well on fructose and galactose. There was little change in pH of various carbon source media during growth of *P. strasseri.*

Pathogenicity tests.—Plants used in pathogenicity tests were obtained by placing tip cuttings of peppermint in flats of sand. After 1 to 2 weeks, the cuttings rooted; and each rooted cutting was transplanted to a pot of nonsterile soil. The plants grew in soil for 1 week or more before inoculation.

Observations indicated that infection by *P. strasseri* often occurred through wounds. Infection and disease severity were tested, using inoculation without wounding, inoculation with incision wounding, and inoculation with puncture wounding. The inoculum, consisting of a piece of culture medium containing spores and mycelia of the fungus, was placed on stems about 3 cm above the soil. The stem was wrapped at the inoculation point with cheesecloth and cellophane tape; then two drops of sterile distilled water were applied to the cheesecloth to keep the inoculation area moist. The plants were kept on a greenhouse bench at 20 to 24 C. Natural daylight was supplemented by fluorescent lights to provide a 16-hr day-length.

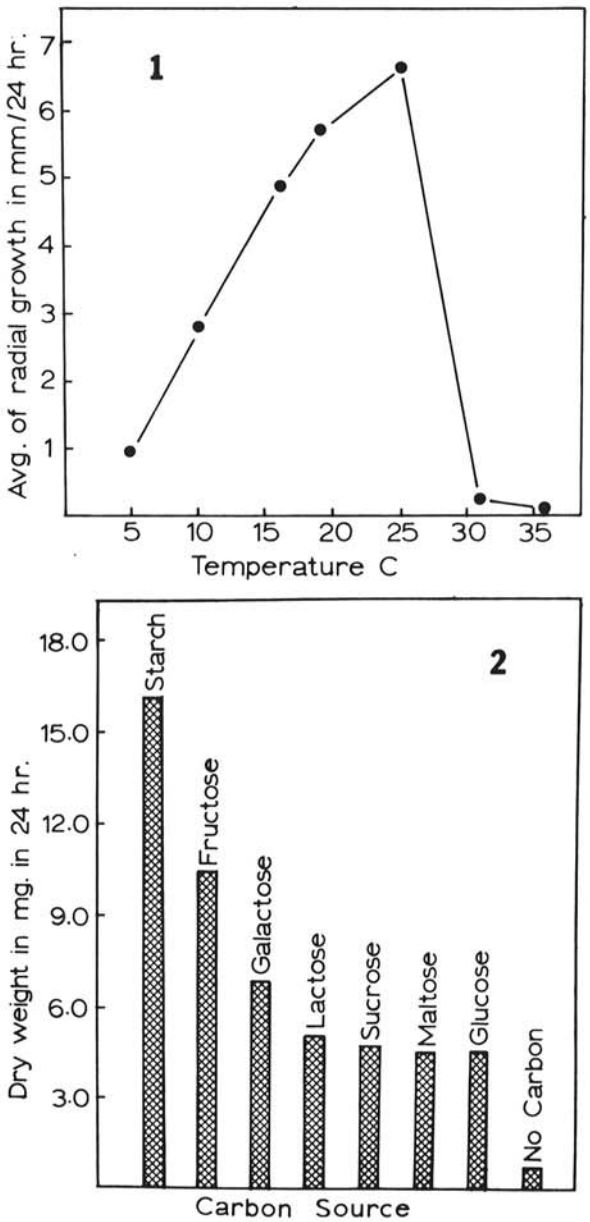

Fig. 1-2. 1) Effect of temperature on radial growth of *Phoma strasseri* on Czapek-Dox agar. 2) Growth of *Phoma strasseri* on various carbon sources. Data represent the average of four replicates of each three harvests at 8, 14, and 18 days after inoculation.

Disease severity was assessed by a numerical disease rating scheme. The numbers 0, 1, 2, 3, and 4 designated no disease, light, moderate, severe, and death, respectively. Dry weights at harvest also were used to determine disease severity. Plants were cut at the soil level and dried in an oven at 105 C for 48 hr, then weighed.

The disease developed most rapidly in plants which were inoculated in incision and stem puncture wounds (Table 1). Disease development was slowest

TABLE 1. Effect of different methods of inoculation with *Phoma strasseri* on disease severity in peppermint plants

Inoculation[a] methods	Disease rating[b] at indicated days after inoculation					Dry wt at harvest (g)
	7	10	13	16	21	
A	2.8	3.4	3.4	3.5	3.7	0.39 ± 0.12[c]
B	1.6	2.2	2.3	2.5	2.7	0.59 ± 0.14
C	2.5	3.0	3.4	3.6	3.6	0.53 ± 0.16
D	0.0	0.0	0.0	0.0	0.0	1.38 ± 0.30
E	0.0	0.0	0.0	0.0	0.0	1.44 ± 0.15

[a]Inoculation methods: A = inoculation with incision wounding; B = inoculation without wounding; C = inoculation with stem puncture; D = noninoculated wounded control; E = noninoculated nonwounded control.

[b]Disease rating: 0 = no disease; 1 to 4 = light, moderate, severe, and dead, respectively.

[c]Standard deviation.

in plants inoculated without wounding, but wounds were not necessary for infection. Dry weights of all the stem-inoculated plants were greatly reduced, as compared with noninoculated control plants (Table 1).

Root inoculation was accomplished by dipping rooted cuttings in a spore suspension containing 100,000 spores/ml and planting each cutting in a pot of untreated soil. The first foliar symptoms were red veins on the leaves, starting with the lower leaves, then spreading to the entire plant. The plants became semiwilted, then recovered. General stunting occurred, and dry weights of infected plants were reduced by 65%, as compared with noninoculated controls. Roots of inoculated plants showed a general necrosis. *Phoma strasseri* was isolated from the roots of inoculated plants, but not from control plants.

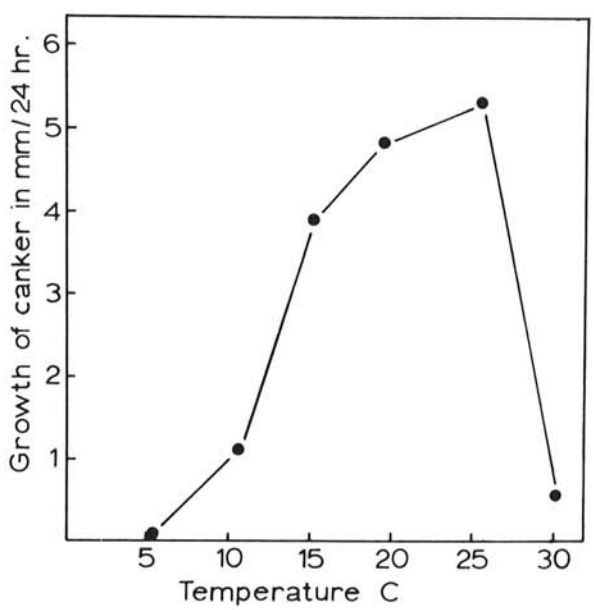

Fig. 3. Effect of temperature on length of cankers caused by *Phoma strasseri* on rhizome tips of peppermint plants.

TABLE 2. Effect of plant age at time of inoculation with *Phoma strasseri* on disease severity on peppermint plants

Plant age (days)	Disease rating at indicated days after inoculation[a]												Dry wt at harvest (g)
	3	4	5	6	7	8	9	10	12	13	14	16	
28	0.6	1.5	2.4	2.8		3.1	3.1			3.4	3.8	3.8	0.24 ± 0.08[b]
34			0.7	1.8		2.4	2.7		3.4		3.4	3.4	0.53 ± 0.1
41					0.9	1.7	2.4	2.4	2.7		3.2	3.2	0.88 ± 0.17
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.33 ± 1.2

[a]Average of 10 plants, disease rating: 0 = no disease; 1 to 4 = light, moderate, severe, and dead, respectively.
[b]Standard deviation.

Temperature and disease development.—Development of the disease on mint rhizome tips was tested at 5, 10, 15, 19, 25, and 30 C. Glass test tubes 25 × 200 mm were filled one-fifth full with white, washed sand saturated with Hoagland's nutrient solution (2). Tubes were then plugged and autoclaved for 15 min at 15 psi. Rhizome tips were punctured near the middle with a needle; then *P. strasseri* inoculum (spores and mycelia) was applied to the wound, and the inoculated rhizome was placed in a glass tube. The tubes were placed in incubators at different temperatures, and length of lesions was measured at 3, 4, and 5 days after inoculation. Rhizome rot developed rapidly over a broad temperature range, 15 to 25 C. Rot was most rapid at about 25 C. Cankers had developed 3 days after incubation at all temperatures tested, except 5 C (Fig. 3).

Effect of plant age at time of inoculation on disease severity.—Plants of different ages (28, 34, and 41 days after cuttings were taken) were inoculated by scratching the stem and applying spores and mycelia of *P. strasseri*. Tolerance of peppermint plants to the effects of *P. strasseri* increased with age (Table 2). Also, dry weights at harvest of plants inoculated when they were 28 days old was greatly reduced compared with plants in the other treatments.

DISCUSSION.—Results of our tests show that *P. strasseri* has no unusual nutritional requirement, and can be grown on media with a variety of carbon sources. Optimum temperatures for growth in culture were similar to other species of *Phoma* (7, 8).

Disease development on peppermint stems was favored by massive wounding, but such wounds were not prerequisite for infection. Bean & Wilcoxson (1) obtained more lesions when Carborundum was used to wound alfalfa stems inoculated with *P. herbarum* var. *medicaginis*. In the field, we have repeatedly observed that *P. strasseri* infection commonly occurs in wounds caused by mechanical cultivation. During greenhouse propagation of peppermint shoot tip cuttings, infection often occurred at the cut ends or where leaves were removed (3).

Rhizome rot is the most serious aspect of the disease, and losses of 50% in rhizome production have been observed in the field. However, we have not yet related rhizome losses to yield losses. Young rhizome tips are especially prone to infection and rapid development of rot. Rapid development of necrosis followed by complete collapse of tissue when young peppermint plants were inoculated with *P. strasseri* has been attributed to the production of pectolytic and macerating enzymes in vivo and in vitro by the fungus (6). At 20 to 25 C, necrosis of rhizomes progressed at an average rate of 5 mm/day (Fig. 3).

As plants mature, their tissues become less susceptible (Table 2). On older plants, infection is usually confined to the soft tissues external to the vascular ring, and takes the form of distinct surface cankers.

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