Effect of Soil Temperature and Host Maturity on Infection of Carrot by Rhizoctonia solani

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ABSTRACT

Carrots grown in soil at various temperatures developed severe crown rot and cavity spot at 20, 24, and 28 C when inoculated with *Rhizoctonia solani*, but little infection occurred at 16 C. When greenhouse-grown carrots were inoculated with *R. solani* at 5- and 7-day intervals 8-45 days after seeding, severe crown rot and cavity spot occurred. Older carrots, both in the field and in the greenhouse, were as susceptible to *R. solani* as the young seedlings. Anatomical studies on the tap root of carrots inoculated at various ages showed that the fungus rapidly colonized the cortex. On young seedlings, prior to cambial division, the fungus penetrated the endodermis and entered the vascular system. On older seedlings, ingress of the hyphae was limited by the cambium.

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Studies on carrot crown rot and cavity spot incited by *Rhizoctonia solani* Kuehn have shown that crown rot first appeared in the field about 1 month after seeding, whereas the cavity spot symptoms were initially observed on 10- to 12-week-old plants (14). When carrots were seeded into naturally and artificially infested muck soil under greenhouse temperatures (24-28 C), severe damping-off occurred. Damping-off, however, was not observed in the field. Carrots are seeded on Wisconsin muck farms at the end of April, but the crown rot phase of the disease is evident first in early June. These observations suggested that low soil temperatures may be limiting the activity of *R. solani* in the month following seeding.

The effect of soil temperature upon the activity of *R. solani* has been documented by several workers (1, 9, 11, 12, 13, 16, 17, 18, 21). Sanford (18) reported little difference in the pathogenicity of *R. solani* on potato stems at 16 and 23 C, whereas Bateman & Dimock (1) found that poinsettias were not damaged below 17 C. Since *R. solani* isolates frequently differ in their response to temperature regimes, the effect of temperature upon the growth and pathogenicity of *R. solani* isolate R-3, known to incite carrot crown rot and cavity spot (14), was investigated.

Studies on the *Rhizoctonia* disease of bean, *Phaseolus vulgaris* L., by Bateman & Lumsden (2) indicated that seedlings were highly susceptible during the first 2 weeks of plant growth. Resistance, however, increased during the 3rd week, and was complete on 4-week-old plants. Shephard & Wood (19) stated that lettuce and cauliflower seedlings became resistant to *R. solani* 4-6 weeks after seeding. Resistance in cauliflower seedlings was attributed to the development of a layer of cells with thickened walls between the cortex and the vascular tissue of the hypocotyl. In order to ascertain whether carrots continued to be infected throughout the growing season or whether crown rot and cavity spot lesions at the time of harvest were the result of infections during the early seedling stages, we investigated the effect of carrot maturity upon the development of crown rot and cavity spot.

The distribution of *R. solani* hyphae in the hypocotyl tissue of carrots inoculated at different ages was studied.

MATERIALS AND METHODS.–Isolates R-1 and R-3 of *R. solani*, both pathogenic to carrot (14), were used in these experiments. Both isolates were identified by R. T. Sherwood as *R. solani* belonging to anastomosis group AG-2 (15). *Daucus carota* L. Chantenay was used in all experiments.

Temperature studies.–Four Wisconsin soil temperature tanks (9) were adjusted to 16, 20, 24, and 28 C. Four crocks, 16.5 cm in diam x 18 cm deep, were placed in each tank. At the bottom of each crock were layered 2 cm of pea gravel upon which 3.5 mm mesh wire screen supported a single layer of cheesecloth. A drainage tube from the base of each crock passed through a stopper in the bottom of the tank. Muck soil treated with methyl bromide (Dowfume MC 2, Dow Chemical Co.) at the rate of 454 g/182 kg soil was used for this study.

Two methods of inoculation were used in separate experiments. In the first experiment, *R. solani* isolate R-3 was cultured on 3% (w/w) cornmeal-sand (CM-sand) medium for 17 days at 22 C. Then 200 g inoculum were evenly spread over the cheesecloth, and the crocks were filled with muck and seeded. Control crocks received 200 g autoclaved CM-sand. Ten days after seeding, carrots were thinned to 10 plants/crock. The plants were irrigated with Hoagland's solution at weekly intervals. Supplementary illumination was provided by banks of 40-w "Gro-Lux" and cool-white Sylvania fluorescent lights, on a 16-hr photoperiod, suspended 60 cm above the crocks. The experiment was harvested 50 days after seeding, and roots and crowns of surviving carrots were rated for lesion development. In the second experiment, six crocks were placed in each temperature tank and we prepared the inoculum by growing isolate R-3 on autoclaved corn kernels in 1-liter Erlenmeyer flasks for 14 days at 22 C. Carrots were thinned to 10 seedlings/crock 15 days after seeding

and inoculated by insertion of a colonized corn kernel about 0.5 cm from each seedling. Control crocks received autoclaved kernels. The experiment was harvested 65 days after seeding.

The effect of temperature upon the growth of isolate R-3 was studied in liquid still culture. Petri dishes containing 15 ml/plate of glucose-asparagine medium (7) supplemented with 0.25 mg/liter thiamine hydrochloride and 0.5 μg/liter d-biotin (GAT medium) were seeded with plugs taken from the edge of 4-day-old colonies on GAT agar using a 7-mm diam cork borer. The GAT medium was adjusted to pH 6.3 with 6 N NaOH before autoclaving. Plates were incubated at 16, 20, 24, and 28 C, and mats were removed at 2-day intervals, up to 10 days, washed on tared Whatman No. 2 filter paper, dried at 80 C for 24 hr, and weighed.

Maturity and anatomical studies.—In one series, carrots were seeded at weekly intervals for 6 weeks in 6-inch clay pots containing equal volumes of steamed muck (1 hr at 100 C) and vermiculite (Terra Lite brand). Prior to inoculation, plants were thinned to 10 seedlings/pot and inoculated by the corn kernel technique. Ten days after the last planting, all the seedlings were inoculated with isolate R-1. The age of the plants at this time was 10, 17, 24, 31, 38, and 45 days. Additional pots were inoculated at each weekly interval in order to obtain samples for anatomical studies. In a second series, isolate R-3 was used to inoculate carrots 8, 13, 18, 23, 28, and 33 days after seeding. Pots were placed in the greenhouse at 24-30 C, and both series were harvested 34 days after inoculation. In order to inoculate carrots prior to emergence, isolate R-3 was grown for 6 days on thin pads of glass wool in 14-cm petri dishes containing 15 ml GAT medium. The washed mats were placed on steamed muck in clay pots. Moist cheesecloth on which carrot seeds had been germinated for 4 days was placed over the mats and covered with muck soil. Six days later, seedlings were sampled, fixed, dehydrated, sectioned, and stained for observation with a light microscope (14).

To ascertain whether carrots in the field are susceptible to *R. solani* isolate R-3 during maturation, about 300 Nantes carrots on a commercial muck farm were inoculated each week by the corn kernel technique 12, 13, 14, and 15 weeks after seeding. Carrots taken from the plots at weekly intervals after inoculation were inspected for lesion development.

RESULTS.—*Temperature studies.*—When CM-sand was used for inoculum, carrots growing at 28 C began to die from crown rot 22 days after seeding, whereas at 20 and 24 C, carrots began to die after 36 days. By 50 days, there were no survivors in the 20-, 24-, and 28-C tanks, whereas at 16 C, only one carrot showed slight crown rot and cavity spot (Table 1). More carrots were infected at 16 C when colonized corn kernels were used as inoculum (Table 1) and the severity of cavity spot increased with temperature. Control plants were symptomless.

On GAT medium, isolate R-3 attained maximum mycelial mat weight after 6 days' growth at 28 C (Fig. 1). At 20 and 24 C, the highest mat weight occurred after 8 days, whereas at 16 C there was a pronounced lag in growth rate for the first 6 days.

Maturity and anatomical studies.—Carrots inoculated at various ages with isolates R-1 and R-3 were equally susceptible to *R. solani* (Table 2). Crown rot was uniformly high at all ages of inoculation, whereas cavity spot was more severe on the older carrots. In the field, carrots inoculated 12-15 weeks after seeding all developed crown rot and cavity spot within 14 days of inoculation, whereas controls were free from disease.

Rhizoctonia mycelium could be found in carrots inoculated at various ages (Fig. 2). Transverse and longitudinal sections of 10-day-old tap roots from plants inoculated at the time of radicle emergence (after 4 days' incubation on moist cheesecloth) showed intense fungal colonization of the cortex and stele (Fig. 2-A,B). Fungal hyphae were seen only in xylem vessels when the cortex had totally collapsed. Upward advancement of the hyphae occurred in the outer layers of the cortex (Fig. 2-B) where hyphae followed the junction lines of cortical cells. Transverse sections from 24-day-old tap roots of plants inoculated 10 days after seeding (Fig. 2-C) showed heavy fungal colonization of the cortex. At several points the endodermis had collapsed, and occasionally the pericycle and primary phloem were invaded.

TABLE 1. Percent crown rot and cavity spot on Chantenay carrots inoculated with *Rhizoctonia solani* isolate R-3

	Inoculum							
	Cornmeal/sand				Corn kernel			
	Temp (C)				Temp (C)			
	16	20	24	28	16	20	24	28
Mean survivors/crock	10.0	0.0	0.0	0.0	6.3	3.0	1.7	2.0
Crown rot (%)	5.0[a]	100	100	100	36.7[b]	70.0	84.7	82.7
Cavity spot (%)	5.0[c]				5.0[c]	33.0	40.0	50.0

[a] Percentage based on 10 carrots/crock with two replicates.
[b] Percentage based on 10 carrots/crock with three replicates.
[c] Percentage based on number of surviving carrots at harvest.
Plants inoculated with cornmeal/sand and corn kernels were 50 and 65 days old, respectively, when harvested.

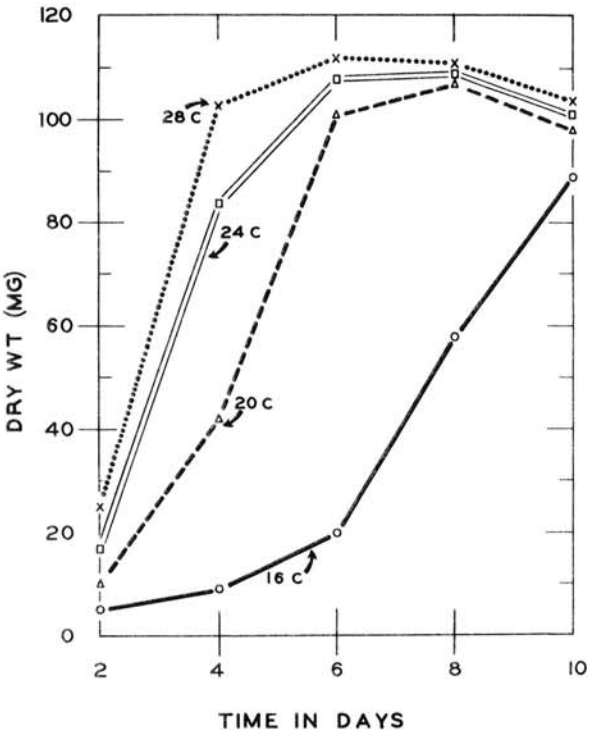

Fig. 1. The effect of temperature upon the growth of *Rhizoctonia solani* isolate R-3 on glucose-asparagine-thiamine medium. Each point represents the mean weight of three mats.

Fungal hyphae were not observed in the cambial zone. The partially sloughed cortex of 39-day-old hypocotyls inoculated 31 days after seeding was densely colonized and at various points the periderm was penetrated (Fig. 2-D). Limited invasion of the secondary phloem occurred, and the phloem parenchyma usually collapsed ahead of the invading hyphae. Hyphae did not enter the cambial zone.

DISCUSSION.—As has been reported for other diseases incited by *R. solani* (1, 13, 17, 21), crown rot and cavity spot of carrots was more severe at high temperatures. The results obtained in the soil temperature studies provided us a basis for explaining our observations from the field, in that the serious effects of the pathogen are not apparent until the soil temperatures have risen above 16 C, which is some time after mid-June in Wisconsin. Although the *Rhizoctonia* isolates used in this study caused "damping-off" of young carrot seedlings in the greenhouse at 24 C, the absence of damping-off in the field may be attributed to low soil temperature during the first 4 weeks of growth. A similar temperature relationship has been reported for the seasonal development of Rhizoctonia canker of alfalfa where root cankers failed to develop at soil temperatures below 20 C (20, 21). Similarly, spinach and sugar beet were unaffected by *Rhizoctonia* damping-off at soil temperatures below 12 C, but were severely attacked at 20-30 C (12).

The continued susceptibility of carrots to crown rot and cavity spot throughout their growth and maturation is in contrast to the response of bean (2), lettuce, and cauliflower (19), which became more resistant with age. The infection of mature carrots by *R. solani* was similar to the mature root phase of *R. solani* on sugar beet (13).

The increase in cavity spot severity on carrots inoculated 1 month or more after seeding may be related to the activity of the inoculum at the time of formation of the horizontal groove (6). If carrot roots are inoculated prior to cambial activity, the cortex, endodermis, and primary phloem and xylem become invaded. This condition was typical of damped-off carrots grown in the greenhouse. When carrots 1 month old or more were inoculated, the fungus rapidly colonized the partially sloughed cortex at the crown and penetrated the periderm. However, the cambial zone was not invaded. On the root, the horizontal grooves were preferentially invaded (14).

TABLE 2. Percent crown rot and cavity spot on carrots inoculated at various ages with *Rhizoctonia solani* and harvested 34 days later

	Age (days) when inoculated					
	10	17	24	31	38	45
			Isolate R-1			
Mean survivors/pot	4.2	3.7	1.7	1.2	3.0	1.5
Crown rot (%)	71.0[a]	77.0	87.0	90.0	85.0	92.0
Cavity spot (%)	18.0[c]	40.0	28.0	40.0	75.0	84.0
			Isolate R-3			
	8	13	18	23	28	33
Mean survivors/pot	1.2	0.6	0.8	0.0	1.8	2.4
Crown rot (%)	95.6[b]	96.4	93.6	100.0	87.6	80.4
Cavity spot (%)	0.0[c]	0.0	0.0		22.2	33.2

[a] Percentage based on 10 carrots/pot with four replicates.
[b] Percentage based on 10 carrots/pot with five replicates.
[c] Percentage based on number of surviving carrots at harvest.

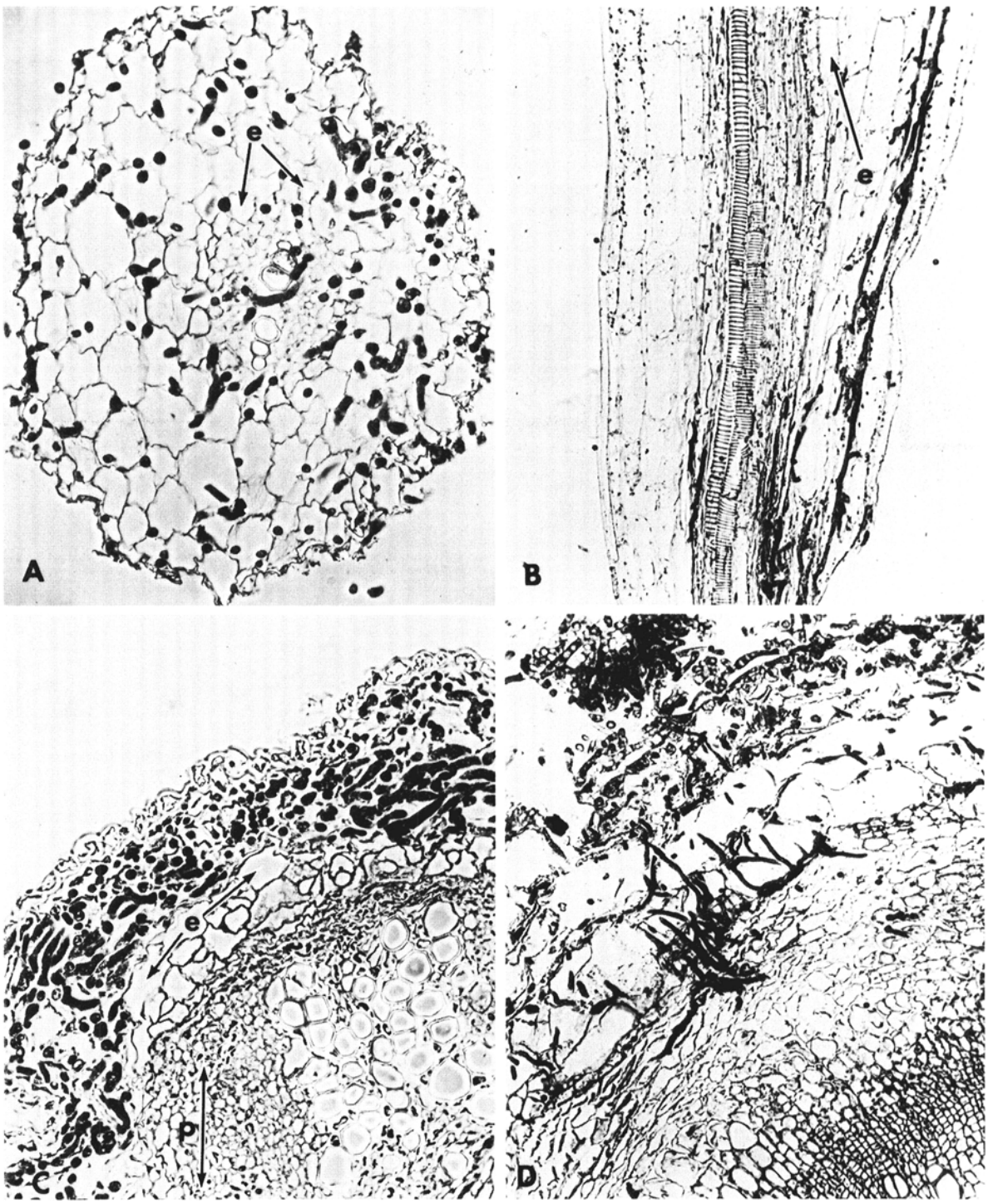

Fig. 2. A) Transverse section of a 10-day-old carrot hypocotyl inoculated with isolate R-3 after 4 days' germination (× 150). **B)** Longitudinal section of a 10-day-old carrot hypocotyl inoculated with isolate R-3 after 4 days' germination (× 59). **C)** Transverse section of a 24-day-old carrot hypocotyl inoculated with *Rhizoctonia solani* isolate R-1 when 10 days old (× 89). **D)** Transverse section of a 39-day-old carrot hypocotyl inoculated with *R. solani* isolate R-1 when 31 days old (× 62). e = endodermis; p = primary phloem.

Although *R. solani* was unable to penetrate the endodermis of bean (3), invasion of the cambium and vascular system occurred on cotton (10), potato (4), and alfalfa (5, 8). On carrot, the ingress of the fungus on older seedlings was halted by the cambium.

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