Pathogenicity to Carrots of Pythium Species from Organic Soils of North America

R. J. Howard, R. G. Pratt, and P. H. Williams

Former Research Assistants and Professor, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706. Present addresses of first and second authors: Alberta Horticultural Research Center, Brooks, Alberta T0J 0J0 and USDA-ARS, Department of Plant Pathology and Weed Science, P.O. Drawer PG, Mississippi State, MI 39762, respectively. Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, University of Wisconsin, Madison.

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ABSTRACT

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Carrot seedlings were grown in samples of organic soils from nine carrot-growing regions of North America at a uniform moisture tension. Pre-emergence damping-off and root dieback were evaluated, and *Pythium* spp. were isolated from diseased roots. Twelve species were identified among 518 *Pythium* isolates. *Pythium irregulare, P. sulcatum,* and *P. sylvaticum* were isolated most frequently and were each found in soils from six or more regions. *Pythium irregulare* and *P. sulcatum* caused severe root dieback in artificially infested soils, whereas *P. sylvaticum* and most other species caused only slight root dieback. Most species caused severe pre-emergence damping-off. Root dieback occurred in soils from all regions and was greater in samples from fields in Wisconsin that had been cropped to carrots than from virgin fields. Root dieback was correlated with the presence of *P. irregulare* and *P. sulcatum* but was not consistently correlated with total *Pythium* populations in soils. Results of this study indicate that the etiology of carrot root dieback is likely to be similar in organic soils throughout North America, and that *P. irregulare* and *P. sulcatum* are the primary causal agents.

Additional key words: Pythium root dieback.

Root diseases of carrots with symptoms of discolored lateral roots, and necrotic, stunted, or forked taproots, have been reported from several regions of North America where carrots are cropped intensively on organic soils. These diseases have been variously described as lateral root dieback (16), brown root (6), rusty root (1, 3, 12, 13), and Pythium root dieback (4, 13, 16). *Pythium* spp. have been implicated as causal organisms of these diseases and also are reported to cause damping-off of carrot seedlings (4, 6) and rot of stored carrots (11).

Symptoms described for the Pythium root diseases of carrots in North America are indistinct and overlapping, and taxonomic standards used by investigators to determine identities of *Pythium* spp. from carrots have not always been the same. As a result, it is uncertain whether these diseases are similar or distinct, whether they are caused by the same primary species or combinations of species in different areas of the continent, or whether they are localized or widespread in distribution. This study was undertaken, therefore, for the purposes of: (i) determining the identity, frequency, and pathogenicity of *Pythium* spp. associated with diseased roots of carrots grown in samples of organic soils from diverse carrot-growing areas of North America, and (ii) determining relationships between disease potentials, occurrence of select species, and total populations of *Pythium* spp. in these soils. From similarities of disease symptoms and severity, and in the distribution and frequency of the two most pathogenic species in soils from diverse areas, it appears that the etiology of Pythium root diseases of carrots is likely to be similar in organic soils throughout North America.

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MATERIALS AND METHODS

Collection of soil samples and determination of disease potentials.—One hundred and forty-seven samples of cultivated and virgin organic soils were collected from the upper 15-20 cm of 66 fields in nine major areas of carrot production in the USA and Canada (Table 1). For each sample, portions of soil taken with a soil tube or hand trowel from three to 10 scattered locations in a field, were composited and stored in sealed plastic bags at 10 C for up to 6 mo prior to assays. Samples from different depths were taken with a soil auger.

Disease potentials of soil samples were determined by measuring the severity of pre-emergence damping-off and of root dieback of carrot seedlings grown in the soils under conditions of uniform moisture tension. Moisture

tension columns (10.0 × 11.5 cm) (8) were filled with similar quantities of soil, packed with equal pressure and planted 1 cm deep with 50 Waltham Hi-Color carrot seeds of known germinability. Reservoirs of deionized water, connected to lower ends of soil columns beneath porous ceramic plates, were first raised to saturate the soil and then maintained at 20 cm below the plates to provide a soil moisture tension of about 20 millibars. Seedlings were grown in the columns at 20 C with 19,000 lux of cool-white fluorescent and incandescent illumination on a 12-hr photoperiod. After 10 days, emerged seedlings were counted and thinned to 10 per column. After 21 days, the soil was saturated and removed from the columns. Seedlings were washed free of soil and the mean percentages of root dieback were determined from visual estimates of 10 seedlings for each soil sample.

Isolation, identification, and pathogenicity of Pythium Spp.—*Pythium* spp. isolates obtained from carrots were used to assay soil columns. Six to 12 sections of diseased roots (each 2-5 mm in length) from one to 10 plants from each soil sample were washed for 2-3 hr in running tapwater, rinsed in sterile water, and plated on Ocana and Tsao's medium (7) or on cornmeal agar (CMA) plus pimaricin (50 μg/ml) and sodium novobiocin (50 μg/ml). After incubation of plates at 20-26 C for 24-48 hr, portions from margins of *Pythium* colonies were transferred to CMA slants for incubation at room temperature for 7-14 days, and then stored at 10 C.

Isolates were identified from reproductive structures produced on CMA, potato-dextrose agar, Schmitthenner's medium (14), and on infested grass blades in water (14) according to the key of Waterhouse (14) and by reference to original and secondary species descriptions (5, 10, 15). Identity of *P. sylvaticum* isolates was verified by their mating reactions with the type cultures (2) on Schmitthenner's medium.

Pathogenicity of *Pythium* isolates was determined by growing carrot seedlings in artificially infested soil in columns under the same conditions used to test naturally infested soil samples. To infest soil, cornmeal-sand medium (9), inoculated with single isolates of each *Pythium* sp. and incubated for 2 wk at 20-24 C, was mixed with steamed muck (organic soil) (1:4, v/v) and incubated for one additional week with daily mixing. Then each *Pythium* population was quantified by dilution plating, and the infested mixture was diluted with additional steamed muck soil to give 1,000 propagules/g. The soil mixtures were then added to columns and sown with carrot seeds. After 10 days, emerged seedlings were counted and thinned to 10 per column, and after 21 days roots were washed free of soil and dieback was evaluated. Two isolates of each species (except for single isolates of *P. afertile* and *P. splendens*) were tested individually for pathogenicity using four soil columns for each isolate.

Pythium propagule densities.—The total *Pythium* spp. population in each soil sample was determined by dilution plating on Ocana and Tsao's selective medium (7). One-ml aliquots of soil, diluted (1:100, 1:50, and 1:25 by weight) in 0.25% water agar amended with 45,000 IU penicillin-G/liter, were removed from stirred suspensions, seeded on plates of medium (three plates per dilution per soil), and evenly spread over the agar surface with a bent glass rod. Seeded suspensions were removed by washing after incubating the plates for 24 hr at 20 C. *Pythium* sp. colonies in the agar were determined from numbers of colonies per gram equivalent of oven-dried soil.

RESULTS

Disease potentials of soils.—Pre-emergence damping-off of carrot seedlings occurred in all samples of soil from all areas, including both virgin and cropped soils in Wisconsin (Table 1). Only slight differences in mean incidence of pre-emergence damping-off occurred between soils in seven of the nine areas sampled. Dieback of roots of carrot seedlings occurred in one or more samples of soil from all areas, and mean severity of dieback differed significantly between some areas. In Wisconsin soils, greater dieback occurred in samples from fields cropped to carrots than in samples from virgin fields. However, in some samples from cropped fields in Wisconsin and other areas, no root dieback was observed. Both pre-emergence damping-off and root dieback were

TABLE 1. Incidence of pre-emergence damping-off and severity of root dieback in carrot seedlings grown in samples of organic soils from nine carrot-producing areas of North America

Condition of soil	Location	No. of samples	Carrot pre-emergence damping-off (%)[a]		Carrot root dieback (%)[a]	
			Range	Mean	Range	Mean
Cultivated	British Columbia	6	42-77	57	0-55	29
	Florida	6	40-82	60	3-48	31
	Michigan	2	52-96	63	0-13	5
	Minnesota	9	50-92	82	0-43	13
	New York	3	44-72	52	0-25	7
	North Carolina	4	...	...	20-25	22
	Ontario	4	54-88	67	0-35	23
	Quebec	8	36-84	61	0-73	30
	Wisconsin	83	38-100	63	0-100	17
Virgin	Wisconsin	22	16-78	64	0-30	7

[a]Damping-off and root dieback were determined with carrot seedlings grown in samples of soil in moisture tension columns with matric water potentials maintained at approximately −0.02 bars. Pre-emergence damping-off was determined from stand counts 10 days after planting of 50 seeds of known germinability in each soil; root dieback was determined from areas of roots visibly browned in 10 seedlings in each soil 21 days after planting.

similar in samples from nearly all depths down to 120 cm in each of six Wisconsin fields.

Identity, frequency, and pathogenicity of Pythium isolates.—Twelve species were identified among 518 *Pythium* isolates collected from diseased roots of carrots grown in soil from the nine regions (Table 2). Forty-two isolates could not be identified because no sex organs or zoospores were produced.

Pythium irregulare, P. sulcatum, and *P. sylvaticum* were isolated most frequently and were each found in soils from six or more areas (Table 2). The other nine species were found in soils from one to four areas. *Pythium irregulare* and *P. sulcatum* caused greatest root dieback in carrot seedlings grown in artificially infested soil, whereas *P. sylvaticum* and most of the other species caused only slight dieback. *Pythium irregulare* and *P. paroecandrum* caused greatest pre-emergence damping-off, but several other species also caused severe damping-off (Table 2).

Pythium irregulare and *P. sulcatum* caused root dieback in carrot seedlings at inoculum densities of 50-1,000 propagules/g soil (Table 3). Severity of dieback generally increased with increasing densities of both species. At inoculum densities greater than 1,000 propagules/g soil, damping-off of seedlings approached 100% and prevented further determinations of root dieback.

Pythium propagule densities in soils.—Total *Pythium* propagule densities ranged from 51 to 9,086 propagules/g in the samples of soil from the nine areas. Propagule densities were similar in 105 samples from virgin fields and fields cropped to carrots in Wisconsin and were not correlated with differences in severity of root dieback. However, among 42 samples of soil from areas outside of Wisconsin, total *Pythium* propagule densities were significantly correlated ($P = 0.001$) with severity of root dieback.

TABLE 2. Identity, distribution, and pathogenicity of *Pythium* spp. isolated from diseased roots of carrots grown in soil samples from nine carrot-producing areas of North America

Pythium species	Distribution and number of isolates[a]									Total isolates (%)	Pre-emergence damping-off[b]	Root dieback (%)[b]
	B.C.	Fla.	Mich.	Minn.	N.Y.	N.C.	Ont.	Que.	Wis.			
P. afertile	0	0	0	0	0	0	0	0	1	0.2	72	0
P. coloratum	3	6	0	6	0	0	0	0	7	4.2	66	3
P. irregulare	16	17	0	0	1	3	7	5	81	25.0	96	86
P. mamillatum	0	0	0	0	0	0	1	0	4	1.0	50	5
P. paroecandrum	6	2	0	0	0	0	0	0	11	3.7	94	39
P. periilum	0	2	0	1	0	0	0	0	0	0.6	58	8
P. spinosum	0	1	0	0	0	4	0	0	0	1.0	88	36
P. splendens	0	0	0	0	0	0	0	0	1	0.2	42	10
P. sulcatum	3	11	0	0	1	1	0	28	77	23.3	80	60
P. sylvaticum	3	2	7	7	0	0	3	12	118	29.3	72	2
P. ultimum	6	0	7	0	0	0	0	0	0	2.5	54	5
P. vexans	0	1	0	0	0	0	0	1	2	0.8	28	0
Nonclassified	4	6	3	1	0	0	2	4	22	8.1		
Total isolates	41	48	17	15	2	8	13	50	324			

[a]Pythium isolates obtained from diseased roots of carrots grown in samples of soil in moisture tension columns at −0.02 bars matric water potential.

[b]Mean incidence of pre-emergence damping-off and severity of root dieback in carrot seedlings grown in artificially infested soil in moisture tension columns at −0.02 bars matric water potential. Two isolates of each species (except for single isolates of *P. afertile* and *P. splendens*) were tested individually for pathogenicity using four columns per isolate.

TABLE 3. Pre-emergence damping-off and root dieback of carrot seedlings incited by *Pythium irregulare* and *P. sulcatum* in artificially infested soil at five inoculum levels

Inoculum density (propagules/g)[a]	Pre-emergence damping-off (%)[b]		Root dieback (%)[b]	
	P. irregulare	*P. sulcatum*	*P. irregulare*	*P. sulcatum*
0	27 A	27 A	0 A	0 A
100	91 CD	57 B	3 A	25 B
500	96 D	48 B	51 B	28 B
1000	77 BC	52 B	30 B	50 C
5000	100 E	99 C	100 C	100 D

[a]Infested cornmeal-sand medium was mixed with steamed muck soil and inoculum densities were adjusted with additional steamed muck following quantification of propagules by dilution plating after incubation for 1 wk.

[b]Mean incidence of damping-off and severity of root dieback in carrot seedlings grown in soil in moisture tension columns at −0.02 bars matric water potential. Numbers within a column not followed by the same letter are significantly different ($P = 0.05$) as determined by analysis of variance and by a LSD test.

DISCUSSION

Pythium root dieback occurred in carrot seedlings grown in samples of organic soils from nine carrot-producing areas of North America under similar moisture conditions. Whenever dieback was severe, one or both of the two most pathogenic species, *P. irregulare* and *P. sulcatum,* were isolated from diseased roots. The frequent occurrence of these highly virulent species in organic soils from diverse areas of the continent suggests that they are likely to be the primary causal agents of Pythium root dieback of carrots grown on organic soil throughout North America. The etiology of this disease, therefore, appears to be simple rather than complex, and the various *Pythium*-induced root diseases of carrots described from other areas (1, 3, 4, 6, 10, 12, 13, 16) may be similar or identical to the root dieback observed in this study.

Propagule densities of total *Pythium* spp. were similar in virgin and cultivated soils in Wisconsin and were not correlated with differences in severity of root dieback in carrot seedlings grown in those soils. This further suggests that the disease is caused by a small or select, rather than large or general, group of *Pythium* spp.

The most severe dieback of roots of carrot seedlings occurred in samples of soil from British Columbia, Florida, and Quebec (Table 1). Both *P. irregulare* and *P. sulcatum* were isolated from seedlings grown in those soils, and these species together comprised at least 48% of all *Pythium* isolates (Table 2). Conversely, little dieback occurred in seedlings grown in soils from Michigan and Minnesota, and neither *P. irregulare* nor *P. sulcatum* was isolated from those seedlings. In one exceptional instance, little root dieback was observed in soils from New York even though both *P. irregulare* and *P. sulcatum* were present. It is possible that inoculum levels of these species in New York soils were too low to cause severe dieback. Barr and Kemp (1) also reported that *P. sulcatum* occurs in some soils in Ontario in which carrot root disease is mild or absent; they suggested that other agents may have to be present in soil to predispose carrots to infection.

In this study, carrot root dieback was only observed in seedlings grown in moisture tension columns for soils from all areas other than Wisconsin. In Wisconsin soils, however, dieback was observed in seedlings both in the field and in columns, and symptoms and severity of disease was similar in the two situations. Both *P. irregulare* and *P. sulcatum* were isolated from diseased carrots from the field in Wisconsin (6, and author's, *unpublished*) and from seedlings grown in the moisture tension columns. These similarities in disease in Wisconsin soils suggest that the root dieback which occurred in seedlings grown in soils from other areas in moisture tension columns also may be similar to disease which occurs in the field.

Pythium irregulare and *P. sulcatum* induced similar root dieback symptoms in carrot seedlings. Severity of dieback caused by these two species also was similar at inoculum levels of 500-1,000 propagules/g soil (Table 3). Either species, therefore, appears capable of functioning as the primary causal agent in the carrot root dieback disease. It is not known whether the two species cause disease independently in the field, or whether they also may function together as primary agents in a disease complex.

Most other species of *Pythium* obtained in this study were isolated infrequently, were only found in soils from one to four areas, and were avirulent or only slightly virulent on established carrot seedlings (Table 2). These results suggest that these species do not function in disease complexes, but rather are only secondary invaders of diseased roots. *Pythium sylvaticum* was isolated frequently from roots of seedlings grown in soils from seven of the nine areas. However, the fact that single isolates of *P. sylvaticum* caused only slight carrot root dieback suggests that this species may also be only a secondary invader of dead or weakened carrot roots.

LITERATURE CITED

1. BARR, D. J. S., and W. C. KEMP. 1955. Olpidium brassicae, tobacco necrosis virus, and Pythium spp. in relation to rusty root of carrots in Ontario and Quebec. Can Plant Dis. Surv. 55:77-82.
2. CAMPBELL, W. A., and F. F. HENDRIX, JR. 1967. A new heterothallic Pythium from southern United States. Mycologia 59:274-278.
3. KALU, N. N., J. C. SUTTON, and O. VAARTAJA. 1976. Pythium spp. associated with root dieback of carrots in Ontario. Can. J. Plant Sci. 56:555-561.
4. MC ELROY, F. D., H. S. PEPIN, and D. J. ORMROD. 1971. Dieback of carrot roots caused by Pythium debaryanum. Phytopathology 61:586-587.
5. MIDDLETON, J. T. 1943. The taxonomy, host range, and geographic distribution of the genus Pythium. Mem. Torrey Bot. Club 20:1-171.
6. MILDENHALL, J. P., R. G. PRATT, P. H. WILLIAMS, and J. E. MITCHELL. 1971. Pythium brown root and forking of muck-grown carrots. Plant Dis. Rep. 55:536-540.
7. OCANA, G., and P. H. TSAO. 1966. A selective agar medium for the direct isolation and enumeration of Phytophthora in soil. Phytopathology 56:893 (Abstr.).
8. PALLAS, J. E., JR., A. R. BERTRAND, D. G. HARRIS, C. B. ELKINS, and C. L. PARKS. 1965. Research in plant transpiration. U.S. Dep. Agric. Prod. Rep. 87. 56 p.
9. PAPAVIZAS, G. C., and W. A. AYRES. 1965. Virulence, host range, and pectolytic enzymes of single basidiospore isolates of Rhizoctonia practicola and Rhizoctonia solani. Phytopathology 55:111-116.
10. PRATT, R. G., and J. E. MITCHELL. 1973. A new species of Pythium from Wisconsin and Florida isolated from carrots. Can. J. Bot. 51:333-339.
11. RADER, W. E. 1952. Diseases of stored carrots in New York State. N.Y. Agric. Exp. Stn. (Cornell) Bull. 889. 64 p.
12. SUTTON, J. C. 1973. Fungi associated with the rusty root disorder of muck-grown carrots in Ontario. Can. Plant Dis. Surv. 53:157-160.
13. SUTTON, J. C. 1975. Pythium spp. produce rusty root of carrots in Ontario. Can. J. Plant Sci. 55:139-143.
14. WATERHOUSE, G. M. 1967. Key to Pythium Pringsheim. Commonw. Mycol. Inst. Mycol. Paper 109. 15 p.
15. WATERHOUSE, G. M. 1968. The genus Pythium Pringsheim. Diagnoses (or descriptions) and figures from the original papers. Commonw. Mycol. Inst. Mycol. Pap. 110. 50 p.
16. WISBEY, B. D., R. J. COPEMAN, and T. A. BLACK. 1977. Other incitants associated with Pythium root dieback of muck-grown carrots in British Columbia. Can. J. Plant Sci. 57:235-241.